Blood glucose response during cardiopulmonary exercise testing in individuals with type 1 diabetes

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Declarations

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed BENJAMIN WELLMAN

Date 05.08.2022

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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Abstract

To characterise blood glucose responses in cardiopulmonary exercise testing (CPET) in individuals with type 1 diabetes and to assess the impact of hyperglycaemia vs euglycemia on physiological and metabolic responses during CPET in T1DM.

This study was a retrospective, secondary analysis of pooled CPET data from three randomised controlled trials using identical 1-minute incremental test stages. Participants followed standardised glycaemic management criteria prior to exercise. During CPX testing, cardiopulmonary variables were measured continuously whilst blood glucose (BG) and lactate values were obtained minutely via capillary earlobe sampling. Anaerobic threshold (AT) was determined using the modified V-slope index by the ventilatory equivalents for CO_2 and O_2 (VE/VCO_2, VE/VCO_2), expressed as a function of VO_2. Data were reported as mean±SD and analysed by a students paired t-test with $p \leq 0.05$.

Data from 36 individuals with T1DM HbA1c 7.3±1.1%, age 32±13 years, diabetes duration 17±10 years, maximum heart rate 180±13 bpm and V̇O2max of 3.2±0.9 $L.min^{-1}$ were included. BG values remained equivalent to sitting concentrations (8.91±2.47 $mmol.L^{-1}$) at each stage of the CPET protocol. The change in BG above the anaerobic threshold was differently significantly from the change in BG below the AT (Sit–AT; +0.2±0.7 vs. AT-Peak; -0.5±0.8 $mmol.L^{-1}$, [i.e. Δ 0.7], $p<0.001$). The rate of change (ROC) between values showed that the change in the ROC from Sit-AT to AT-Peak was significantly different between groups (Sit-AT; Δ0.004±0.06 vs. AT-Peak; Δ-0.049±0.10 $mmol.L^{-1}$, $p=0.01$). Between Hyperglycaemia (Hyper) vs. Euglycemia (Eu), BG remained comparable within each respective starting BG levels. The magnitude of change in BG from Sit-AT was significantly different between groups (Hyper Δ-0.09±0.35 vs. Eu Δ+0.32±0.76 $p=0.030$). Hyper from Sit-AT had a significantly different magnitude in the ROC to AT-Peak (-0.04±-0.06 $mmol.L^{-1}$/min, $p=0.032$) but not in the Eu group.

We found that under correct CPET protocol, glycaemia is minimally disturbed and does not result in hypoglycaemia or changes in performance outcomes. However, starting exercise with high blood glucose levels (hyperglycaemia) impacts some cardio-metabolic outcomes during maximal and recovery phases around CPET.

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Abbreviations

ACSM – American College Sport Medicine

ADA – American Diabetes Association

AGE – Advanced Glycation End

AMPK – AMP- Activated Protein Kinase

AT – Anaerobic Threshold

BG – Blood Glucose

CHO – Carbohydrate

CO2 – Carbon Dioxide

CPDP – Centres for Disease Control and Prevention

CPET – Cardiopulmonary Exercise Testing

CVD – Cardiovascular Disease Control and Prevention

DAG – Diacylglycerol

DCCT – Diabetes Control and Complications Trial

DKA – Diabetic Ketoacidosis

FFA – Free Fatty Acids

G-6-P – Glucose-6-Phosphate

GH – Growth Hormone

GI – Glycaemic Index

GL – Glycaemic Load

HAAF – Hypoglycaemic Associated Autonomic Failure

HIIT – High Intensity Interval Training

HR – Heart Rate

HU – Hypoglycaemic Unawareness

IDeg – Insulin Degludec

IGlar – Insulin Glargine

IR – Insulin Receptor

IRS – Insulin Receptor Substrate

LGI – Low Glycaemic Index

MDI – Multiple Daily Injections

NO – Nitric Oxide

NPH – Neutral Protamine Hagedorn

O2 – Oxygen

OS – Oxidative Stress

PETCO2 – Patient End Tidal Carbon Dioxide

PETO2 – Patient End Tidal Oxygen

PFK – Protein Kinase

PKC – Protein Kinase C

RER- Respiratory Exchange Ratio

RPM – Revolutions Per Minute

T1DM – Type 1 Diabetes Mellitus

T2DM – Type 2 Diabetes Melitus

WHO – World Health Organisation

Chapter 1 – Introduction and Literature Review

Literature Review

1.1. Introduction

Type 1 diabetes mellitus (T1DM) is characterised by progressive autoimmune destruction of beta cells (β-Cells) of the pancreas, specifically the islets of Langerhans, resulting to a complete or relative deficiency in insulin formation and secretion. This results in hyperglycaemia and a potential to develop diabetic ketoacidosis (DKA); DKA is a consequence of a deficiency in insulin, therefore, greater fat breakdown increases serum free fatty acids that are then metabolised producing large quantities of ketones and metabolic acidosis. Fluid depletion is a serious problem caused by osmotic diuresis in DKA. A degree of loss in α-cell function, can lead to altered release of glucagon, which is a peptide hormone that functions to increase glucose concentrations if glucose levels are low within the circulation. These deficiencies lead to a reliance on another counter-regulatory mechanism release of catecholamines from the adrenal medulla; the outcome of a loss of this can result in hypoglycaemia unawareness, the magnitude in hypoglycaemia can vary, but can be serious and sometimes even fatal in the short and long term (Verberne, A. J. et al 2016).

The difficulty in management of exogenous insulin levels around exercise can make the individual susceptible to significant variations in blood glucose levels, which can lead to hypoglycaemia (blood glucose levels <3.9 $mmol.L^{-1}$) (American Diabetes Association, 2005), or hyperglycaemia (blood glucose levels >7.0 $mmol.L^{-1}$ when fasting, and glucose levels >11 $mmol.L^{-1}$ 2 hours after meals) (American Diabetes Association, 2014). A consistent variation in blood glucose levels can increase the risk of acute or chronic health comorbidities.

Despite practical interventions to improve glycemia around exercise, there is a certain complexity in the detail around certain factors around exercise. This would include the duration of the exercise, how frequent the exercise is done through a certain period, and the type of intensity the exercise is maintained at, which all have a dynamic in how glycemia responds. These variables result in a reduction in participation in exercise for T1DM individuals, due to the lack of education in the area which is why it is of high importance the understanding and practise of this thesis is needed.

Cardiopulmonary Exercise Testing (CPET) is an important test usually used to clinically evaluate individuals with underlying exertional symptoms that range from respiratory and cardiovascular disorders. CPET provides useful information about the cardiovascular, respiratory, neurosensory, and metabolic stress that occurs during the graded exercise test. Given that diabetes can impact cardio-vascular function, exploration of the person with type 1 diabetes responses to CPET is warranted. Therefore, this can help develop into a better understanding in the use of physical activity for the targeted treatment and management of people with type 1 diabetes. With the combination of blood glucose sampling and CPET, data can help us understand the physiological mechanisms that occur under physical stress.

There is very little research which represents the detailed continuous blood glucose monitoring in CPET in T1DM and not used as the primary outcome of the studies, as they investigate understanding the consequence of poor glycaemic control and resultant exercise performance around CPET such as oxygen economy (Moser, O et al 2017), heart rate dynamics during CPET (Moser et al, 2018), and the consequence of hyperglycaemic and euglycemic conditions during exercise (Stettler et al, 2006). The assessment of glucose monitoring in these studies were used for safety protocol or to maintain a certain glycaemic level for investigation purposes. Also, due to the variations in the type of protocol within these studies, this can demonstrate different physiological responses and are problematic in comparison. Therefore, the confounding purpose for our study is to fully visualise a minute-by-minute response in blood glucose during a progressive intensity ramp protocol procedure to understand the response of glycemia under a increase in physical stress until exhaustion. Information presented such as this can be a beneficial insight of CPET and help clinicians better picture the importance of standardising blood glucose within certain ranges, which is before the assessment, during the exercise period, and the post exercise period of people with type 1 diabetes mellitus (T1DM).

Based on that stated, the two main aims of this thesis were to:

Aim 1: Characterise blood glucose responses in CPET in individuals with type 1 diabetes.

Aim 2: Assess the impact of hyperglycaemia vs euglycemia on physiological and metabolic responses during CPET in T1DM.

1.2. Type 1 Diabetes

In 2019, approximately 537 million adults (aged 20-79 years) were living with diabetes, that is 1 in 15 of the world's adult population (International Diabetes Federation, 2021). Recent trajectories indicate by 2045 the rise will increase the 700 million (Saeedi et al, 2019). With childhood diabetes on the rise with more than 1.1 million children and adolescents are living with type 1 diabetes (T1DM) and globally, 128,900 new cases diagnosed every year (International Diabetes Federation, 2011).

Within the UK, since 1996, the number of diagnosed type 1 diabetes cases has risen from 1.4 million to 3.5 million; by 2035 more than 5 million people will be diagnosed in England with T1DM. Whilst type 2 diabetes (T2DM) is more widespread relative to type 1, despite this T1DM is still on the rise in diagnosing individuals with type 1, mainly children (Diabetes UK, 2015); in 2015, more than 542,000 children in the world were living with T1DM (Diabetes UK, 2015). There is no single underlying cause for T1DM, however, there are several factors that have been proposed to cause/trigger the development, these can start with genetic predisposition to be more susceptible to T1DM. however, despite having a genetic predisposition, it is more likely to be cause by environmental factors such as viral infection, types of vaccines, Low levels of vitamin D, or a consistent increase in the demand of insulin (Diabetes UK, 2019).

Within the UK, both type 1 and 2 diabetes produce a financial burden on the economy. The NHS spends £10 billion a year on diabetes (10% of its entire budget) and are on the course to reach 16.9 billion by 2035 (Diabetes UK, 2012). A significant amount is spent on diabetes care, and the associated complications. People with diabetes are two and half more times likely to have a heart attack or experience heart failure, with more than 2,000 cases of heart failure every week, due to a lack of blood glucose control (Diabetes UK, 2019). Despite these costs, if there was a more efficient and effective way for diagnosis and management of symptoms of these comorbidities, this could help reduce the further cost of treatment to individuals and stop the risk of further health complications.

1.3. Blood Glucose Regulation in T1DM

Type 1 diabetes is a metabolic disorder which results from an autoimmune response that destroys the β-Cells in the pancreas, in hand resulting in a loss in the secretory function in insulin. This lack of control of insulin secretion plays an integral role in the difficulty in the management of blood glucose levels, therefore the pharmacological replacement of exogenous insulin is needed to help with the fluctuation of blood glucose concentrations throughout the day, via multiple daily injections (MDI) or insulin pump. Several counter-regulatory hormones are responsive to ensure euglycemia is achieved in non-diabetic individuals between 4.0-7.0 mmol.L^{-1} (Diabetes UK, 2000), however people with T1DM have a daily struggle to keep the blood glucose levels euglycemic. The process, which translates the stimulation of hormone secretion is the glucose-stimulated β-cell insulin release (Ashcroft, et al 1994). Insulin prevents glucose output from the liver by the inhibition of gluconeogenesis and glycogenolysis (Figure 1) (Mandarino, 1981, Saltiel, & Kahn, 2001). Insulin enables the insulin-dependent uptake of glucose via Glucose transporter type 4 (GLUT 4) translocation (Leto & Saltiel, 2012) into skeletal muscle and adipose tissues, therefore removing exogenous glucose from the bloodstream (Khan & Pessin, 2002).

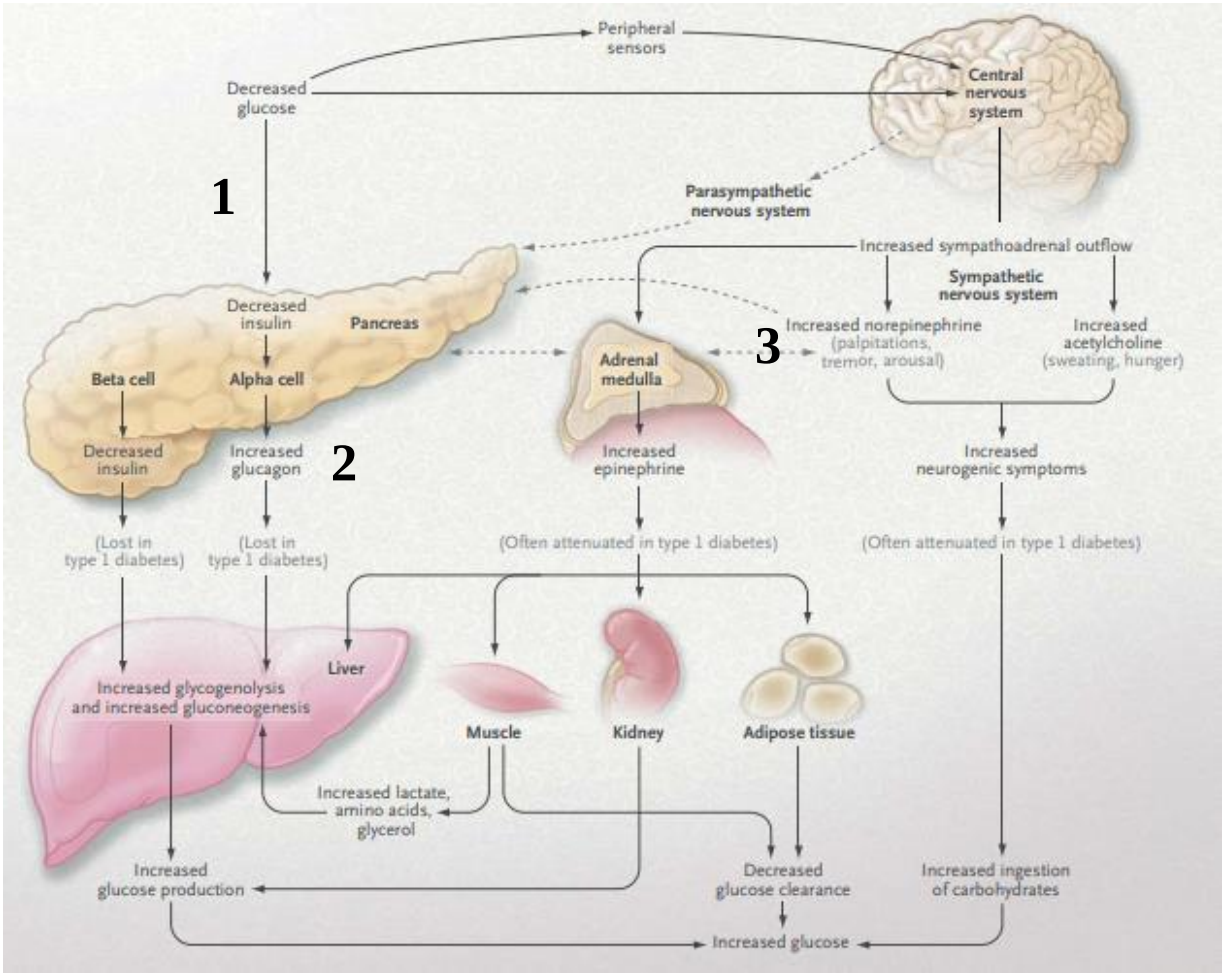

Figure 1 Physiological responses to a decrease in blood glucose concentrations. Modified from Cryer, P. E. (2013). 1, 2 and 3 represent the order in which the response occurs when blood glucose concentrations are dropping.

When blood glucose concentrations are low (3.9 mmol.L^{-1}) (Desouza et al, 2010) insulin is then repressed and glucagon and catecholamines (adrenaline, noradrenaline) are released. Catecholamines, such as adrenaline released through β_2-adrenergic receptors, in hand stimulates glycogenolysis and gluconeogenesis (Deibert & DeFronzo, 1980), this can be critical when glucagon is impaired in T1DM (Rizza, R. A., Cryer & Gerich, 1979). Growth Hormone (GH) is released and suppresses Phosphatidylinositol-3-Kinase (P13K) (Moller, L et al 2009), which is reliant by the insulin-dependent cellular response of glucose, therefore suppressing GLUT-4 activation (Kim & Park, 2017). Furthermore, cortisol increases the movement of fatty acids and amino acids to the liver, therefore promoting gluconeogenesis (Khani & Tayek, 2001 & Ghanaat. Tayek 2005). If there are remnants in exogenous insulin existsing in T1DM this proposes a high exposure to a consequent lead into hypoglycaemia.

1.3.1. Hyperglycaemia

Hyperglycaemia is caused from an increasingly high level in blood glucose concentrations (>11 mmol.L^{-1}; NICE, 2016), with blood glucose levels >7.0 mmol.L^{-1} when fasting, and glucose levels >11 mmol.L^{-1} 2 hours after meals (American Diabetes Association, 2014) this is due to a lack of circulating insulin or can be due to other factors such as foods high in carbohydrate or have a high glycaemic index or load (Diabetes UK, 2019). High-intensity exercise has also shown to increase blood glucose concentrations during exercise (Fahey et al, 2012). Furthermore, within nocturnal periods in T1DM has shown to have a likelihood to have a high-level concentration in blood glucose, primarily due to the secretion in hormonal changes which impair the action of insulin or produce hepatic glucose production, this is called the '*Dawn Phenomenon'* (Diabetes UK, 2019). Studies have emphasised the importance of the nocturnal spike of GH which increases hepatic glucose production, this is due to a decrease in insulin sensitivity in T1DM (Perriello et al, 1990, Møller & Jørgensen, 2009). Diabetes characteristics such as the duration of diabetes, and the higher levels of HbA1c, which have been proposed as characteristics to create a greater influence in the magnitude of hormonal flux during nocturnal periods (Perriello et al, 1991).

In relation to HbA1c, this helps provide evidence about an individual's average glucose levels during the previous two to three months, which is the half life of a red blood cell (McPherson & Pincus, 2021). This is formulated by the glycation of haemoglobin blood cell protein, which is a nonenzymatic reaction between glucose and part of the haemoglobin protein, this is part or a normal function cycle, however, the more glucose available, the more glycated haemoglobin will be present in one period (Sherwani et al, 2016), which is why HbA1c is used a diagnostic and prognostic biomarker to help understand an individual's glycemia.

Having a long-term consistency in high blood glucose concentrations can present acute comorbidities for individuals with T1DM. High increases in blood glucose can in hand produce ketone bodies (3-ß-

hydroxybutyrate, acetoacetate, and acetone) through Acetyl CoA; if ketone levels rise over 1.6-2.9 $mmol.L^{-1}$ this can decrease blood pH, therefore lead to the development of subsequent metabolic acidosis (Kanikarla-Marie & Jain, 2016). Obvious symptoms for individuals during diabetic ketoacidosis are severe dehydration, vomiting, needing to urinate, due to the high ketone (acidic) in urine, hypotension in some cases can be fatal (Diabetes UK 2019).

Hyperglycaemia can further create direct effects on the body, such as the cardiovascular system. Hyperglycaemia-induced endothelial damage can occur De Vriese, A. S., Verbeuren, T. J., Van de (Voorde, Lameire & Vanhoutte, 2000). From the start of the glycation of products, this leads to the activation of the protein kinase (PFK) isoforms via synthesis of diacylglycerol (DAG), increasing the hexosamine pathway flux, therefore creating advanced glycation end (AGE) products (Rolo & Palmeira, 2006). Furthermore, the high glucose concentrations cause oxidative stressors such as H_2O_2 (Hydrogen peroxide), NO (Nitric Oxide), which have been shown to activate Protein Kinase C (PKC) and influence vasodilatory capabilities (Ceriello et al, 2007). DAG-PKC pathway is associated with many vascular abnormalities in the retinal, renal, neural and cardiovascular tissues in diabetes mellitus (Das Evcimen, & King, 2007). DAG-PKC also activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidise; this is a major factor that causes atherosclerosis (Lien, Chen, Tsai & Lin, 2021). Therefore, it is of high importance to maintain euglycaemia and have a control of blood glucose levels in T1DM individuals to be able to perform exercise and activities at optimal levels without any major effects in metabolism.

1.3.2. Hypoglycemia

Iatrogenic hypoglycemia occurs when there are uncharacteristically low blood glucose levels (>3.9 $mmol.L^{-1}$ – American Diabetes Association 2005) which can be caused by exogenous insulin administration or unrecognised or inadequately treated malnutrition (Chittineni et al, 2019). There is a concern when in this state, this is due to the occasional hypoglycemic unawareness (HU), which is defined as the failure of a T2DM and T1DM individual to identify a significant drop in blood glucose concentrations (Martín-Timón & del Cañizo-Gómez, 2015). Thus hypoglycemia, can be symptomatic and asymptomatic. 'Symptomatic' is defined as when plasma glucose levels drop $\leq$3.9 $mmol.L^{-1}$ which goes with physical symptoms such as dizziness, anxiety, and shaking, this can exacerbate into more extreme symptoms like a loss of consciousness, sickness, seizures, coma, or even death. 'Asymptomatic hypoglycemia' is when blood glucose levels are below $\leq$3.9 $mmol.L^{-1}$, however, there are no accompanying symptoms to go with this blood glucose value (American Diabetes Association, 2005).

Causes of hypoglycemia can result from inaccurate exogenous insulin dose or timing, food intake or a bout of physical activity (Shafiee, G. 2012). If too much insulin administered in comparison to the dietary intake, the mediated uptake of glucose, besides the inhibition of hepatic glucose production and a compromised glucose-regulation (Cryer, 1994; Figure 1), leads to a fall in blood glucose. Individuals who are unaware of recurrent hypoglycemia can worsen counter-regulatory responses and experience low glycaemic thresholds (Cryer, 2003), this is a result from hypoglycemia-associated autonomic failure (HAAF) (Cryer, 2010), and this can result in failure to recover from such events of hypoglycemia. This reduction in glucagon response is due to the blood-to-brain response, as the brain has an increased glucose transport to sustain cerebral metabolism during hypoglycemia (Cryer, 2005).

Hypoglycemia can be avoided despite hypoglycemic unawareness (HU) by applying the correct hypoglycaemic management and interventions to prevent hypoglycaemic episodes with accurate dietary intake, insulin regime, and exercise management. Organised management of glycaemia and increasing the awareness of hypoglycemia occurrence can produce reversible effects of recurrent hypoglycemia when assessing over a 3-month period (Rattarasarn and Cryer, 1994). However, this is also dependent on the individual's diabetes duration and well-controlled glycaemia (HbA1c) (Reno 2013; Fanelli and Cryer 1999).

1.4. Treatment for T1DM

It has been shown that tightening glycaemia through the correct dosage of exogenous insulin (insulin therapy), alongside the frequent analysis of blood glucose with appropriate dietary choices can reduce complications associated with T1DM. The Diabetes Control and Complications Trial (DCCT) demonstrated this with using insulin therapy such as an external insulin pump or three or more daily injections, and by using continuous glucose monitoring (CGM). This intervention aimed to tightly control blood glucose levels to normal ranges and successfully delayed the occurrence of diabetic neuropathy, nephropathy, and retinopathy in T1DM (DCCT Research Group. 1990). There has been updated literature of this study over a 30-year standpoint that consolidates the effectiveness of the intervention in T1DM for improving the prospects for a healthy life span (Nathan et al, 2014). More recent studies demonstrate the use of CGM (Yeh, Brown, Maruthur, 2012) and different types exercise training interventions (Tonoli et al, 2012) with correct insulin administration and dietary guidance (Zafar et al, 2019) can significantly improve glycaemic control.

Continuous glucose monitoring as part of a glycaemic management strategy demonstrated the benefits in metabolic control and a reduction in HbA1c by 1% over a 5 day period (Ziegler et al 2011). With the improvement in glucose monitoring technology, monitoring systems shows how manageable and discrete interstitial fluid glucose concentrations can be. Flash systems and CGM's allow for more present readings of glucose concentrations, as CGM technology allows 5-minute readings of blood glucose concentrations by scanning the 'flash read' device. The American Diabetes Association (ADA) recommend people with T1DM to stay around HbA1c levels of 7% and preferably below 6.5%, which can be achieved with avoidance of hypoglycaemic episodes or any other effects from treatments (American Diabetes Association, Hunt 2008). CGM's have demonstrated the capability to reduce HbA1c and blood glucose concentrations and show a relationship in a reduction in hypoglycaemic occurrence with improved glucose control (Oliver and Gimenez, 2020). Individuals at higher HbA_{1c} concentrations show the most significant change to the targeted range over a 3-6-month period (Deiss et al 2006, JDRF Continuous Glucose Monitoring Study Group, 2009). However, individuals who already have a normal HbA1c range can still have a small, or minimal change in their HbA_{1c} levels (Pickup 2011).

1.4.1. Bolus Insulin

Bolus insulin is usually taken with a meal. The use of a bolus insulin is due to its fast-acting response for the disposal of postprandial glucose and can be done by either taking a a short acting insulin (Human Regular) or rapid-acting insulin (Insulin Aspart, Insulin Fiasp, Insulin Glulisine, and Insulin Lispro).

Insulin Aspart (IAsp, NovoRapid^{TM}, Novo Nordisk A/S) and Lispro (Eli Lilly and Company) are fast-acting bolus insulins that are administered with a meal to help mimic a normal insulin secretory response in healthy individuals, and dynamics and pharmacokinetics are seen as comparable in T1DM (Plank et al 2012). This provides a much faster onset to postprandial glucose control, and long-term effects, such as showing improvements in HbA1c levels and has association in a reduction in the risk of hypoglycaemia in T1DM individuals compared to regular human insulins (Hermansen et al 2004, Home, Lindholm & Riis, 2000). The use of fast acting bolus insulin has been adapted into clinical exercise trials, which has been looked at on its own through comparisons between different insulin dialogs to assess the efficacy of glucose disappearance or absorption rate during exercise periods (Bode et al 2017, Home et al 2006, Yamakita et al 2002). Also manipulated with a basal therapy by an educational intervention and self-management to optimise the use of insulin therapies, by combining the two insulin therapies together to inform participants on dosage requirements around feeding stages, exercise sessions, and nocturnal periods to create a tight glycemia throughout daily exercise or physical activities (Heller et al 2012, Rabasa-Lhoret, Bourque, Ducros & Chiasson, 2001). Other studies look further into adjustments around exercise for premeal insulin dose reduction and postprandial exercise of different intensities and durations, treated intensively with a basal-bolus insulin regimen (ultralente-lispro), and separately with dietary adjustments (Campbell et al 2015). Further outcomes of some studies have suggested a reduction in bolus insulin when consuming a pre-exercise meal, can reduce the risk of exercise induce hypoglycaemia post-exercise (Eckstein et al, 2020).

1.4.2. Basal Insulin

Basal Insulin is a longer-lasting insulin intended for a slow release throughout the day. Certain insulin dialogs such as Degludec (IDeg) and Glargine (IGlar) have demonstrated to produce lower glucose variability throughout the day consistently each day (Heise et al 2012, Ratner et al 2000, Home et al, 2004) and has shown to reduce the risk of hypoglycaemia and improvements in HbA1c (Ratner et al 2013, Monami, Marchionni & Mannucci, 2009). Due to the differences in the dynamics and pharmacokinetics of each basal analogue, they can produce differences in the magnitude of glycaemic control. Insulins such as IDeg, present lower rates of nocturnal hypoglycaemic episodes when compared to other basal insulins (Mathieu et al 2013). Clinical trials over a 2-year period have demonstrated a reduction in total daily dose (-9%) of insulin when using IDeg when comparing against IGlar (Heller et al 2013), which has been discussed due to the ultra-long action profile and provided a

less variable glucose-lowering effect from each administration and showed consistency (Heise et al 2012).

Recent studies have demonstrated the relevance of the manipulation of basal insulin in preparation of exercise, having a 25% reduction in basal insulin leads to more time spent in euglycemia, and a reduction in the time spent in hypo- and hyperglycaemia (Moser et al 2019).

Furthermore, basil insulins have been used within clinical trials such as Moser *et al* (2019), to understand the need for manipulating post prandial periods pre-exercise and reductions (25% reduction compared to a normal 100% administration) in basil insulins and what effect this has during exercise on blood glucose levels. These studies investigated changed in glycemia during parts of the exercise period, and the effect this has on risk of nocturnal glycemia post exercise.

Also, studies that distinguished the effect between different basal insulins like IGlar (insulin glargine) showed there was stronger evidence for IDeg versus IGlar in reducing the rates of hypoglycaemic events (Ratner et al, 2013), which demonstrated different dynamics of each basal analogue and the efficacy in the glucose lowering effect. Some note should also be made of dietary intake and basal manipulation around regular exercise, which suggests that a low GI food pre-exercise has been shown to be superior to bolus insulin reduction (50%) for prevention of hypoglycaemia during exercise in people with type 1 diabetes. (Ekstein et al,2020). This validates the importance of manipulation of insulins and dietary intake with basal insulins, which can produce beneficial control of glycaemia during and post-exercise.

1.5. Dietary Intake

Dietary intake plays a pivotal role in the control in T1DM management and goes hand-in-hand with insulin and exercise. A lack of understanding in carbohydrate intake to insulin dosage can leave individuals with T1DM at high risk to hypoglycaemic events. Consuming carbohydrate of a high glycaemic index/load (GI/GL) and/or taking an inaccurate amount of circulating insulin from a carbohydrate load can result in hypoglycaemia. Therefore, intake of low-GI foods, has been proposed to improve HbA1c levels without the increased risk of hypoglycaemia (Gilbertson et al 2001) and to improve insulin sensitivity (Marsh et al 2011). Therefore, applying the correct dietary approach to individuals with T1DM and providing nutritional education can help improve glycaemia and reduce the risk of glycaemic events.

Furthermore, carbohydrate counting, allows individuals with T1DM to adjust bolus insulin units to the varying carbohydrate content (Bell et al., 2014). This allows greater flexibility in their diets and reduction in blood glucose variability and has reported a reduction in HbA1c after 3 months (Mehta et al 2009). T1DM individuals need to understand their target glycaemia range (euglycemia) in response to different meals and the duration and onset of their insulin regimes.

1.5.1. Exogenous insulin administration

The uptake of exogenous insulin is a vital element in the handling in glucose in people with T1DM. The principal behind insulin therapies is to aim to mirror the secretory pattern found healthy individuals (Figure 2) and maintain euglycemia throughout 24 hours; this done with the combination of a basal-bolus regime.

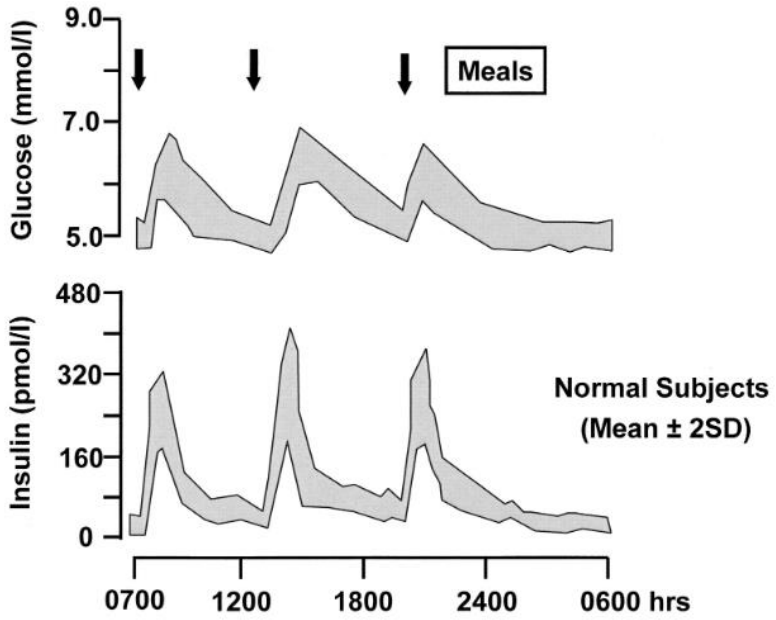

Figure 2: Normal insulin secretion response throughout a 24-hour period. The figure shows pancreatic insulin secretion due to secretory bursts in response to mealtime feeding in healthy subjects. (from Rossetti et al 2008).

The basal insulin injected in the morning or the evening, is dependent on the individual's regime and the type of basal analogue used, which are all designed to produce consistent effect over a 24-hour period. Bolus insulin is injected with every meal; this is done to produce an artificial imitation of normal insulin secretion in healthy individuals. However, with the variability of different type of insulin analogues present different physiological profiles, therefore different responses (Table 2).

Along with the differences in the pharmacokinetics of each type of insulin, there are other biological factors which can affect the absorption kinetics. Injection site can determine the onset of action, the abdomen has been shown to be a reliable site for consistent rate of absorption compared to the administration at the thigh (Süsstrunk et al 1982) and the arm (Koivisto and Felig 1980). However, increased fat mass can decrease the rate of absorption (Sindelka et al 1994). Furthermore, an increase in acute exercise can create an increased rate of absorption (Pitt et al 2020), this absorption rate may be due to the increase in body temperature, therefore influencing the rate of blood flow to increase circulating insulin (Lauritzen, Binder, and Faber 1980).

1.6. Physical Exercise in T1DM

1.6.1. Physical Activity Guidelines

The World Health Organisation defines physical activity as 'any bodily movement produced by skeletal muscle (WHO, 2011), whereas exercise is the intentional aim to improve or maintain fitness. In relation to T1DM individuals the participation in physical exercise plays an important role in the management of glycemia.

Health Organisation	Exercise Recommendations
Diabetes UK	Referenced from WHO guidelines: Adults should do at least 150 minutes of MOD or at least 75 min of VIG in a week, or a combination of MOD & VIG.
ACSM	A recommended 20 to 45 minutes exercise at a low intensity of 40-60% VO_2 max for 5-7 days/week, or daily at a low intensity.
CPDP	MOD intensity for >30 minutes for ≥ 5 days of the week. Lower duration of exercise is advised than a longer duration throughout a smaller time span of days.

Table 1. Exercise recommendations for individuals with T1DM. From Diabetes UK: Exercise [article online], (2013); ASCM-American College Sports Medicine (2000); CPDP; Centres for Disease Control and Prevention (2013). MOD: Moderate-intensity exercise, VIG: Vigorous-intensity exercise, VO_2 max: Maximal Aerobic rate.

1.6.2. Glycaemic benefits from Physical Activity

The effects of consistent physical exercise on glycaemic control have been highly discussed. The effect of types of exercise training on glycaemic control demonstrated a reduction in HbA1c levels (-1.6%) in aerobic exercise (Röhling et al, 2016, Salem et al, 2010). However, some studies found no significant reductions during the intervention of aerobic exercise training (Laarkson et al, 2000; Fuchsjäger-Mayrl et al 2002). Despite these discrepancies of the studies, this could be down to certain factors, such as starting HbA1c levels (8.9 ± 1.4; Salem et al, 2010 vs. 7.3 ± 0.2; Fuchsjäger-Mayrl et al 2002) as having a larger variation in glycemia over a month period would create a higher saturation in glycated haemoglobin (Higher HbA1c) before the exercise program, therefore over the intervention period the HbA1c may be reduced at a larger magnitude compared to baseline levels due to the new turnover of new red blood cells after the 6 month trial period.

Furthermore, the design of the study could influence the outcome. Due to the study population size (N=190; Salem et al 2010 vs. N=26; Fuchsjäger-Mayrl et al 2002) which provides much larger confidence in the data and removing the chance of error. Furthermore, within these studies, there was a more detailed progression overload plan within aerobic exercise sessions with professional intervention in Salem *et al* (2010) whereas studies such a Laarkson *et al* (2000) conducted the study only at moderate intensity exercise between 30-60 minutes, which isn't specific to each individual and may not progressively overload each training session. Despite some studies showed no change in HbA1c levels

after an aerobic exercise program, there were other improvements such as a 20% increase in insulin sensitivity over a 12-week period, which is the increase responsiveness of the cells to insulin (Landt et al, 1985), therefore there is the support of a lower bolus insulin dosage (Yki-Järvinen, DeFronzo, & Koivisto, 1984).

Regardless of opposing literature around changes in glycaemia and around aerobic exercise in T1DM, it is reasonable to suggest that taking part in regular exercise has the potential to tighten blood glucose levels during exercise, therefore improve HbA1c levels, but needs to be more thoroughly investigated. However, there are other well-defined benefits in T1DM and should be encouraged (Kennedy et al, 2013).

1.6.3. Barriers to physical exercise in T1DM

Regardless of the benefits that relate to physical activity for those with T1DM, less than 20% of patients performing aerobic exercise more than two times per week and 60% of the patient population performing no kind of physical exercise (Bohn et al, 2015). Four key barriers have been seen as the causal factors towards the low number in participation (i) Fear of hypoglycaemia, (ii) loss of control of their condition, (iii) low fitness levels, and (iv) work schedule. However, it was displayed that strategies and implementation of insulin pharmacokinetics around exercise is needed to help reduce these barriers (Brazeau, Rabasa-Lhoret, Strychar & Mircescu 2008). Therefore, this shows the clinical relevance of this study to help understand application and education into exercise to reduce risk of glycaemic events and barriers around exercise.

Furthermore, T1DM is a significant precursor and comorbidity to cardiovascular disease. Which have shown that there is an increased rate of incidences as individuals with diabetes are 48% more likely to have cardiovascular complications (Diabetes UK, 2012). Which is why research has underpinned specific strategies in aiding and promoting exercise training programmes which provides recommendations in aiding individuals with T1DM to improve their blood glucose control around exercise participation to prevent risks of glycaemic events. These recommendations and strategies in providing information around will in hand help increase the rate of participation (Buckley et al, 2020). As exercise programmes for T1DM has been associated with an improved cardiovascular profile and glycaemic control (decreased daily insulin dosage, and HbA1c) (Wu, Dickinson, Kim, Chua & Warburton, 2019).

Taking into consideration of these barriers, healthcare specialists have underlined the importance in preparation and keeping a record of blood glucose levels. Post-exercise hypoglycaemia is more likely to happen if blood glucose levels are less than ~7mmol.L^{-1}(Riddell et al, 2017). Within the range of 7-10mmol.L^{-1} pre-exercise for aerobic and anaerobic & HIIT (high-intensity interval training) sessions can proceed as a safe level, but with caution (Riddell et al, 2017). Despite these thoroughly and thought-out blood glucose management strategies, the knowledge within the T1DM population of these

strategies and the skills in recognising and treating variations in glycaemia before or after exercise is limited (Diabetes UK 2018). This further establishes the need to interpret strategies for glycaemic control (insulin dose adjustments, carbohydrate ingestion, and starting glucose concentrations) and then translate them to the T1DM population to develop a detailed understanding to reduce the fear of hypoglycaemia and other barriers around physical activity.

1.7. Glucose regulation during aerobic exercise

Sub-maximal exercise, blood glucose maintenance is a balance between glucose appearance from the liver, and glucose disposal from the demand of blood glucose by the working skeletal muscles (Goodwin, 2010), this is done by afferent feedback signals, constituting and feedback mechanism (Wasserman, Shi & Vranic, 2002). This signalling of glucose appearance is caused by the inhibition of insulin secretion and the rise of the hormone glucagon. During exercise, there are multiple metabolic responses, which help regulate the blood glucose, whether it demands an increased appearance or disappearance of blood glucose.

These responses are governed by the endocrine and nervous system, which help to regulate blood glucose responses. When exercise is instigated, an increase in catecholamines is secreted which bind and activate one of the G-protein coupled receptors, the α-adrenergic receptor from outside of the cell (Mangmool, Denkaew, Parichatikanond, & Kurose, 2017). This produces a suppression of insulin secretion due to the increase of norepinephrine (Broadstone, Pfeifer, Bajaj, Stagner, & Samols, 1987). Without the presence of insulin, the liver becomes sensitive to the appearance of glycogen that produces a sustained production of glucose (Wasserman et al 1989). Furthermore, catecholamines can play a critical role in the production of glucose through gluconeogenesis when exercise is prolonged for more than two hours at high intensity, which shows the role in catecholamines to increase time to exhaustion and be a prime regulator of glucose production (Kreisman, Halter, Vranic & Marliss, 2003), therefore due to the increase in glycogenolysis glucose utilization is less than glucose production (Marliss & Vranic, 2002). When exercise is only for a short bout of moderate aerobic exercise, the magnitude in catecholamines is reduced compared to higher intensities. Insulin levels remain stable throughout exercise due to catecholamines preventing the glucose-mediated insulin secretion (Marliss & Vranic, 2002).

Despite insulin being one of the main regulators of glucose uptake through the translocation of GLUT4 transport protein at rest, during exercise, it has been shown that exercise or muscle contraction does not increase tyrosine phosphorylation of the insulin receptor or insulin receptor proteins (Howlett et al, 2002). The interaction between insulin and exercise during aerobic activity is dependent on insulin sensitivity, during exercise there is a decrease in plasma insulin in healthy cohorts due to the increase in sympathetic activation and inhibition on pancreatic insulin release (Richter, Sylow & Hargreaves, 2021). Therefore, although insulin and exercise promote movement of glucose to muscle, they can

oppose each other's effects of fuel mobilization from tissue stores to blood by reducing hepatic glucose production if there is a reduced sensitivity (Richter, Sylow & Hargreaves, 2021).

However, glucose uptake during exercise is still augmented due to obligatory role of skeletal muscle AMP-activated protein kinase (AMPK) in maintaining mitochondrial capacity and contraction-stimulated glucose uptake via GLUT4 translocation (O'Neill et al 2011). Regardless of the suppression of insulin, the appearance of insulin left in the bloodstream contributes to the uptake of glucose to the skeletal muscle (O'Neill 2011).

Therefore, taking into consideration the metabolic effects of aerobic exercise and regulation of blood glucose during this period and the combination of high intensity in CPET we will be able to consolidate the relevance of characterising glucose stability during CPET. By looking at minute by minute intervals and the analysis of the rate of change in blood glucose during exercise will help to fully visualise any metabolic inhibitory response and counterregulatory failure in T1DM.

1.7.1. Fuel Metabolism

During exercise, the type of metabolic pathway used to meet energy demands for the working skeletal muscles is based on the duration and intensity of the exercise and the availability of substrate to determine the choice of fuel metabolism. Low intensity "steady state" exercise (~25%$\dot{V}O_2$ max) plasma Free Fatty Acids (FFA) increase gradually increases and serves as a predominant energy source from adipose tissue; therefore, glycogen breakdown and glycolysis are not stimulated (Romijn et al, 1993). However, when intensity increases at a moderate rate reaching ~ 65% $\dot{V}O_2$ max lipolysis does not increase further (Figure 3) due to fat oxidation rate exceeding maximal amounts that could be obtained by plasma and the maximal energy expenditure (Romijn et al, 2000). During exercise that exceeds around ~85% $\dot{V}O_2$ max, fuel shifts to carbohydrate, and the reliance on fat decreases due to Carbohydrate (CHO) aerobic ATP production yield being more efficient than that from fat (Rauch et al, 1995).

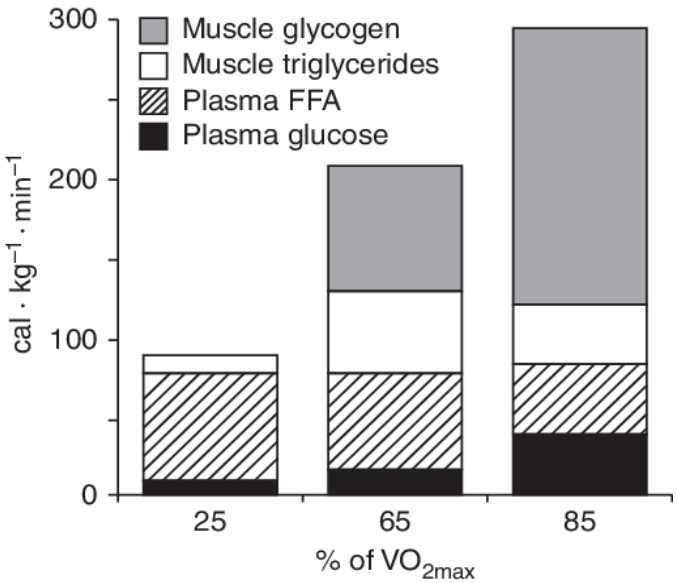

Figure 3: The maximal contribution to energy expenditure derived from FFA and glucose are taken from the blood and the contribution of muscle triglyceride and glycogen stores after 30 min of progressive exercise expressed as a function of exercise intensity % of $\dot{V}O_2$ max (from Romijn et al, 1993).

If exercise duration increases at a moderate "steady-state" intensity (~65% $\dot{V}O_2$ max) for ≥120 minutes, hepatic and skeletal glycogen stores become exhausted, this creates a shift towards increased lipid oxidation and reduced CHO oxidation rates (Hammond, Fell & Morton, 2018). Glycogen is the main source of energy production from the start of "steady state" exercise intensity, therefore constantly reducing availability over a long exercise period and glycogen stores begin to deplete and exercise intensity working capacity decreases (Bergström & Hultman, 1967).

1.7.2. Effects of insulin levels on fuel metabolism

Within the body, an increase in insulin levels may increase glucose uptake to the skeletal muscle, therefore decreasing blood glucose concentrations. Insulin inhibits the process of lipolysis and gluconeogenesis while inhibiting the release of glucagon, and promoting glycogen synthesis (Qaid & Abdelrahman, 2016). However, while exercising at a moderate intensity for a long duration shows an increase in the catecholamine epinephrine and glucagon, therefore increasing the hepatic glycogenolysis, gluconeogenesis, and lipolysis (Wasserman & Cherrington, 1991).

It has been demonstrated with CHO feeding pre-exercise of moderate-intensity (~65% $\dot{V}O_2$ max) that being in a fed state during exercise prevailed higher insulin and blood glucose concentration, this in hand created higher CHO oxidation rates as the primary source of fuel, inhibiting lipolytic activity (Jeukendrup, 2003). However, being in a fasted state (5-6 hours) induces a significant increase in fat oxidation due to the stimulation of lipolysis-stimulating hormones and the limited action of insulin (Vieira, Costa, Macedo, Coconcelli & Kruel, 2016). However, due to the ingestion of CHO pre-exercise, plasma insulin is also higher pre-exercise, therefore during the first 10 minutes of exercise hepatic glycogen output is lower in CHO feeding compared to control, which suggests hyperglycaemia and hyperinsulinemia before exercise can inhibit the feed-forward mechanism of glucose production but still increase CHO oxidation through blood glucose availability (Marmy-Conus, Fabris, Proietto & Hargreaves, 1996). However, this is dependent on the availability of insulin to counteraction effects of catecholamines during exercise.

1.6.3 Managing glycaemic responses from acute exercise in T1DM

When considering glycaemic responses in individuals with T1DM it is important to understand the prescription of exercise, therefore this will allow individuals to enhance engagement in exercise programs and the ability to optimise health benefits. The responses of glycaemia have a complexity around the exercise characteristics of the intensity and duration. Therefore, research around exercise has been targeting exogenous insulin and dietary adjustments around different exercise modalities, which has high importance for individuals with T1DM to ensure safety and optimise performance without risk in glycaemic fluctuation.

1.7. Continuous Exercise

Continuous exercise is normally at low to a moderate intensity below the anaerobic threshold, this exercise modality is well considered to increase the likelihood of hypoglycaemia (Yardley et al, 2013, Briscoe & Davis, 2006). The mechanisms behind the counterregulatory response to hypoglycaemia are hindered in people with T1DM as once they have injected exogenous insulin there is an inability to suppress the insulin administered, this hyperinsulinemia causes a reduction in glucagon sensitivity despite an increase in counterregulatory hormones, therefore creating an imbalance of glucose production to glucose utilization (Ionescu-Tîrgovişte et al, 1980, Zinman et al, 1977). Circulating insulin and muscular work increases insulin-mediated and non-insulin mediated glucose uptake from the blood and into the skeletal muscle, which causes a decrease in blood glucose appearance (Riddell & Perkins 2006). Also, exercise can enhance the increase in insulin absorption rate through temperature increase and blood flow volume. The degree of hypoglycaemic effect in exercise is also affected by the pharmacokinetics of the insulin analogue (Sharma et al, 2019).

Glucose uptake from the non-insulin mediated pathway is from the increase in skeletal muscular contraction. This energy demand of contraction increases the ATP to AMP ratio, which causes the activation of AMP-activation protein kinase (AMPK), this leads to the phosphorylation of TBC1D4 and TBC1D1 (a GTPase activating protein), which is the major influence of glucose uptake (Richter & Hargreaves, 2013). TBC1D4 and TBC1D1 promote the translocation of GLUT4, which allows a larger magnitude in glucose uptake during muscular work (Cartee, 2015).

Insulin-mediated glucose uptake is caused by the increase of circulating insulin, which requires insulin to bind to its receptor on the plasma membrane, the Insulin Receptor (IR), this leads to tyrosine phosphorylation of the receptor, therefore, activating Insulin Receptor Substrate (IRS). This facilitates the phosphorylation and the activation of Akt and in hand stimulates TBC1D4 which is the main precursor of GLUT4 translocation to increase the rate of glucose uptake (Cartee, 2015).

The counter regulatory hormones that respond to hypoglycaemia from the increase in blood glucose uptake are hindered in individuals with T1DM, therefore increasing the likelihood of exercise-induced hypoglycaemia (Galassetti et al, 2003). The exercise stimulated growth hormone and catecholamine response to the increase in glucose uptake fails to respond to compensate the appropriate increases in endogenous glucose production (Diabetes Research in Children Network (DirecNet) Study Group, 2006). Furthermore, it has been shown there is an increased blunting effect that occurs after either antecedent hypoglycaemia or prolonged moderate exercise on subsequent exposure to either stimulus during moderate-intensity aerobic exercise (Galassetti et al, 2003).

After continuous aerobic exercise, individuals with T1DM are open to a higher risk of post-exercise hypoglycaemia and nocturnal hypoglycaemic events 6 to 24 hours post-exercise (Cryer 2010). This is due to the decrease in insulin levels within the blood and with the increase AMPK activity, which responsible for the glucose transport; however, suppressing glycogen levels (Horton & Subauste, 2016). Also, the deactivation of mTOR (mTORC1) which is used to impair insulin signalling down the P13K/Akt pathway (Wu & Garvey, 2010).

Post-exercise there possesses a reduction in counter regulatory hormonal secretion, this creates a higher risk of nocturnal hypoglycaemia. During the recovery phase of a single bout of continuous exercise, the further decline in blood glucose is elicited by the lack of the ability to reduce circulating insulin levels, and the withdrawal or responsiveness of counter regulatory hormones, therefore a continued non-insulin mediated uptake to restore muscle and hepatic glycogen stores (Friedrichsen, Mortensen, Pehmøller, Birk & Wojtaszewski, 2013). The window of the risk of hypoglycaemia can occur up to 31 hours post-exercise, as prior to exercise there have been occurrences of a blunting effect of the appearance of adrenaline and glucose production in response to subsequent hypoglycaemia (Sandoval, Aftab Guy, Richardson, Ertl & Davis, 2006). It has also been shown that during sleep adrenaline levels are severely lowered and only increase by 34.5% whereas when awake it increases up to 557% (Hirotsu et al, 2015). Cortisol also reacts in the same way at night whereas when awake it is increased by 140%, which shows despite the increase in these hormones, which help, promote glycogenolysis, this impaired counter-regulatory hormone response only acts as an inhibitor of glucose appearance (Jones et al, 1998). This shows the long-lasting effect of exercise in T1DM individuals on the susceptibility of hypoglycaemia after a bout of continuous exercise.

1.7.1. Basal Insulin Manipulation

From an understanding in the responses in glucose uptake during exercise from exogenous circulating insulin and therefore a larger glucose uptake, there has been a variety of research in the trying to manipulate dosage pre-exercise with the underlying aim to reduce the risk of hypoglycaemia (West et al, 2010, West et al, 2011). It has been widely recommended to reduce basal insulin pre-exercise to reduce the risk of exercise-induced hypoglycaemia (Campbell et al, 2015, Zaharieva & Riddell, 2017, Pinsker et al, 2016). This has been shown by a reduction of basal insulin by 20% (80% of total daily dose) 45 minutes pre-exercise with reduced post-prandial bolus insulin and a low glycaemic index (LGI) feeding produced higher protection of hypoglycaemia over a 24hour period post-exercise (Campbell et al 2015) (Table 2). Furthermore, using an ultra-long-lasting insulin IDeg and a reduction by 75% total daily dose compared to a full dosage (100% total daily dose) pre-exercise showed that time spent in a euglycemic state was increased when a 25% reduction was implemented around regular exercise and less time spent in hyperglycaemia/hypoglycaemia (Moser et al, 2019). In addition, this is dependent on the type of exogenous basal insulin, and the time-action profile of the basal insulin as a study stated the

effectiveness of the basal insulin, Determir in reducing the risk of exercise-induced hypoglycaemia compared to NPH (Neutral Protamine Hagedorn) insulin and Glargine (Tunis et al, 2009). However, when comparing IGlar and IDeg showed similar responses in the reduction in hypoglycaemic response during and post-exercise at moderate 'steady-state' intensity with a basal-bolus regime (Heise et al, 2016, Hermansen et al, 2004).

1.7.2. Bolus Insulin Manipulation

Due to the lack of ability to reduce circulating insulin during exercise, a rational response to reduce the risk of exercise-induced hypoglycaemia is to reduce prandial bolus insulin prior to exercise. It has been demonstrated that a reduction in pre-meal bolus insulin dosage by either a 25, 50 and predominant number of studies with a 75% reduction has shown to reduce the risk of exercise-induced hypoglycaemia (West et al, 2010, West et al, 2011, Bracken et al, 2011, Campbell et al, 2015). There is a limited amount of research that shows the optimal strategy as there is variation in the time pre-exercise of the bolus dosage and the CHO intake, and there are very few studies that implement a post-exercise bolus dosage time and CHO intake. However, a study by Campbell *et al*, (2013) suggested an optimal strategy to reduce the risk in exercise-induced hypoglycaemia. This showed a 25% pre-exercise dose reduction 60 minutes pre-exercise, with a post-exercise dosage of a 50% reduction 60 minutes after exercise with a CHO meal of $1g.kg^{-1}$. This showed no episodes of acute hypoglycaemia (after 9 hours); however, this did not display any glycaemic responses after this period and did not support late onset hypoglycaemia. However, it has been proposed that during different types of aerobic intensities and durations required the appropriate adjustments in reductions of pre-meal insulin and type of CHO intake (Rabasa-Lhoret, Bourque, Ducros, & Chiasson, 2001). Therefore, there needs to be more research in the detail of bolus insulin dose reductions pre-and post-exercise in the type of aerobic exercise intensity and duration to improve the confidence in a recommendation for individuals with T1DM.

1.7.3. CHO Feeding Strategies

Just as the focus alone on exogenous insulin manipulation, there is also a lack of the combination of exogenous insulin manipulation and CHO feeding strategies to evaluate the effect of glycaemia. However, it has been shown that LGI CHO feeding pre-exercise reduces the rate at which blood glucose increases and improves the responses to exercise (West et al, 2011). This is due to the complexity of the carbohydrate, therefore increasing the time to break down into simple sugars. It has also been proposed post-exercise before night-time to intake a LGI CHO snack with a reduced rapid-acting insulin dose (50% usual) to reduce the risk of night-time hypoglycaemia for 8 hours post-exercise, yet the risk of nocturnal hypoglycaemia beyond this time period remains (Campbell et al, 2014).

Reference	Design	Insulin & CHO	Exercise	Drop in BG (mmol.L^{-1})	Hypoglycemia
West et al, (2010)	-7 T1DM	Unchanged Basal. Rapid-acting meal dose as a % of a full dose: 100% 75% 50% 25%	45 min treadmill run at 70% $\dot{V}O_2$ peak	100%: -6.1 75%: -5.3 50%: -5.5 25%: -3.2	100%: n=3 75%: n=1 50%: n=1 25%: n=1
West et al, (2011)	-7 T1DM	Unchanged Basal. Rapid-acting meal does reduction of 75%. LGI meal consumed prior to exercise at either: 30 min 60 min 90 min 120 min	45 min treadmill run at 70% $\dot{V}O_2$ peak	30min: -4.4 60min: -4.8 90min: -5.7 120min: -6.3	30min: N/A 60min: 1 90min: 2 120min: 5
Rabasa-Lhoret, (2001)	-8 T1DM	Unchanged Basal. Bolus Lispro meal dose as % of full dose: 100% 50% 25%	Cycling at times and intensities of: 30min at 50% $\dot{V}O_2$ max 60min at 25% $\dot{V}O_2$ max 30min at 75% $\dot{V}O_2$ max 60min at 50% $\dot{V}O_2$ max	100% Lispro: 30min at 50% $\dot{V}O_2$ max: -3.4 60min at 25% $\dot{V}O_2$ max: -3.0 30min at 75% $\dot{V}O_2$ max: -3.0 60min at 50% $\dot{V}O_2$ max: Hypo. 50% Lispro: 30min at 50% $\dot{V}O_2$ max: -2.3 60min at 25% $\dot{V}O_2$ max: -3.0 30min at 75% $\dot{V}O_2$ max: N/A 60min at 50% $\dot{V}O_2$ max: -4.2 25% Lispro: 30min at 50% $\dot{V}O_2$ max: N/A 60min at 25% $\dot{V}O_2$ max: -3.0 30min at 75% $\dot{V}O_2$ max: -2.7 60min at 50% $\dot{V}O_2$ max: -3.1	4 episodes of glycaemia occurring at 60min at 50% $\dot{V}O_2$ max under 100% Lispro.

Table 2: Blood glucose responses to acute insulin and dietary adjustments for continuous 'steady-state' exercise in T1DM.

1.8. High-Intensity Exercise

When exercise intensity increases, the oxygen demand increases. When the intensity beyond this point requires a high output of energy where oxygen delivery and the utilization can no longer meet the demand. Therefore, this causes a shift in energy production predominantly from non-oxidative metabolism. This type of energy production is limited and can only be maintained for a short period, this is usually seen in activities such as sprinting or exercises that require short powerful movements.

Differing from low to moderate-intensity aerobic exercise, high-intensity exercise shows an increase in blood glucose, this can but from 10s sprints (Bussau et al, 2006) or a progressive, sustained high-intensity exercise to exhaustion (130% $\dot{V}0_2$ peak) (Harmer et al, 2006), this is also to have a similar response in healthy non-diabetics (Adams, 2013). However, the variation in hyperglycaemia is variated with the intensity and duration of the exercise. During a 12-minute high-intensity exercise until exhaustion elicited a 7-fold increase in glucose production post-exercise, but glucose, utilization was only 4-fold, therefore creating an imbalance of blood glucose appearance in the circulation (Purdon et al, 1993). On the other hand, the 10s sprint, due to the increase in catecholamines, growth hormone, and cortisol there was an increase in hepatic glucose production, however, this was due to the decline in glucose utilization instead a disproportionate increase in glucose production to utilization in consistent high-intensity exercise (Bussau et al, 2006, Bally et al, 2016).

Responses to high-intensity exercise, there have been studies suggesting the increase in the glucoregulatory response of a large shift in the elevation in catecholamine and growth hormone levels, which prevents the decline in blood glucose levels post-exercise (Marliss et al, 1991), however only a small increase in glucagon levels (Sills & Cerny, 1983). It has been proposed that the causation of high blood glucose levels from high-intensity exercise is due to the sympathoadrenal system, which has been said to be a major supporter of liver glucose production during high-intensity exercise (Sigal et al, 1996, Näveri, Kuoppasalmi & HaÈrkoÈnen, 1985). Furthermore, it has been shown that an increase in catecholamines within the circulation oppose the insulin-mediated fall in glycaemia by the inhibition of insulin-mediated glucose uptake in the skeletal muscle (Marliss & Vranic, 2002). A further lack of glucose utilization induced by high-intensity activity is the large burst of glycogen utilization and breakdown to supply glucose to the skeletal muscle, which causes a large increase in glucose-6-phosphate (G-6-P) concentration for mitochondrial respiration, which prevents hexokinase activity due to the large concentration, thereby reducing glucose disappearance (Chen & Gollnick, 1994).

During the early recovery phase post-exercise, catecholamine and growth hormone levels stay elevated which increase a consistent appearance in blood glucose levels for a short period of time (Guelfi, Jones & Fournier, 2005), this accounts for individuals who are non-T1DM and individuals with T1DM.

However, for individuals with T1DM, hyperglycaemia is prolonged post-exercise due to the decreased insulin sensitivity and the reduced insulin secretion into the circulation in response to high blood glucose levels (Riddell & Perkins, 2006).

A study demonstrated the effect of bolus corrections on the effect of post exercise hyperglycaemia during high intensity exercise of 25-minute high intensity interval training (HIIT) for an 8-week period. This showed a reduced risk of hyperglycaemia post-exercise with a Bolus dosage of 100% or 150% of their normal bolus insulin dosage, which was based on the individual's insulin correction factor, this is how much 1 unit of rapid insulin each person will need to lower your blood glucose levels over a 2-to-4-hour period when you are in a pre-meal state. This is based around each of their insulin sensitivity levels (Aronson, Brown & Riddell, 2019). Further studies in assessing the effect of high intensity interval exercise with the adjustment of evening basal dose post-exercise in the afternoon with a 10, 20, and 30% evening basal adjustment. This adjustment in all the basal insulin reductions (10, 20, 30%) showed no association with post exercise related hyperglycaemia, and no significant change in glucose variability to hyperglycaemic levels in each regime (Lee, Way, Johnson & Twigg, 2020). Studies also regarding high intensity exercise in the type of insulin therapy has shown that continuous subcutaneous insulin infusion (CSII) instead of multiple daily injection (MDI) therapy was advantageous as risk of glycaemic variability was higher in MDI (Gawrecki et al, 2017).

There has been a range in literature that demonstrate helpful interventions to help manipulate and manage blood glucose levels pre-exercise, during, and post-exercise in high intensity activity. This can be done by adjusting basal-bolus regimes pre- or post-exercise (Table 3) , or by dietary management (Riddell et al, 2017, Colberg et al, 2016). Therefore, understanding from the review of aerobic exercise and high intensity, the use of CPET and the efficacy of a thought through protocol around insulin adjustment and dietary intervention needs to be assessed to properly characterise the variability of blood glucose during graded exercise into this anaerobic state until exhaustion.

Reference	Design	Insulin & CHO	Exercise	Drop in BG (mmol.L^{-1})	Hypoglycemia
Campbell et al, (2015)	-9 T1DM	50% reduction in bolus insulin (Lispro/Aspart).	45 min treadmill run at 75% $\dot{V}O_2$ max (CON). Or 45 min Games activity stimulation (LIST).	CON: -5.3 ± 0.4 LIST: -1.1 ± 1.4	CON: n=3 LIST: n=2
Bussau et al, (2006)	-7 T1DM	No Adjustments to Bolus/Basal Regime.	10s sprint prior to a 20 min cycle at 40% $\dot{V}O_2$ peak (SPRINT). Or rest prior to 20 min cycle (CON).	SPRINT: -2.9 CON: -2.9 Recovery BG decreased by 1.2 under CON. SPRINT remained the same.	N/A
Guelfi et al, (2005)	-7 T1DM	No Adjustments to Bolus/Basal Regime.	30 min treadmill run at 40% $\dot{V}O_2$ max (MOD) or the same intermittent with 4-sec sprints every 2 min (IHE).	MOD: -4.4 IHE: -2.9 No difference in starting BG	MOD: n=2 IHE: n=1 During exercise
Harmer et al, (2006)	-8 T1DM	Reduced basal dose by 1-2 Units.	Cycling with a 3 min warm-up at 20W, then 110 rpm at power at 130% $\dot{V}O_2$ peak until exhaustion.	BG increased 1 and 3.5 from baseline during exercise and within 60 min after exercise.	N/A

Table 3: Acute blood glucose responses to high intensity exercise in T1DM. BG; Blood Glucose. CHO; Carbohydrate. LIST: Loughborough Intermittent Shuttle Test.

1.9. Cardiopulmonary Exercise Testing

Cardiopulmonary exercise testing (CPET) is a non-invasive, graded exercise-tolerance test, which is used to evaluate the integrative responses of the pulmonary and cardiovascular systems across a graded exercise intensity continuum (Albouaini et al, 2007). A combination of cardiorespiratory and blood metabolite data taken while the test is underway can be used to determine peak aerobic rate and originate individualised submaximal intensities with accuracy for research and rehabilitation programmes. There has been a large increase in the acknowledgement of the efficacy in CPET in evaluating an individual's pathophysiological status and susceptibility to disease (Deborah, Jonathan, Liguori, Magal, 2018, Herdy et al, 2016, Balady et al, 2010)

It has been shown that healthy individuals with T1DM have an attenuated $\dot{V}O_2$ peak uptake (Turinese I, Marinelli P, Bonini M, 2017) and to lower oxygen economy at these metabolic submaximal thresholds (Moser et al, 2018). Furthermore, atypical responses in heart rate dynamics during CPET have been shown in individuals with T1DM (Moser et al, 2018), with a reasonable suggestion that glycaemic control could be an underlining factor in the abnormalities (Moser et al, 2018). Despite the various research in the associations between chronic indices of glycaemia in CPET performance (Solomon et al, 2015), the influence of acute glycaemia on exercise capacity remains unidentified. Research has explored the patterns of fuel metabolism in people with T1DM during continuous moderate exercise showed compared to a euglycemic condition, a hyperglycaemic state creates an increase in tolls of local and systemic CHO oxidation, blood lactate formation and glycogen breakdown yet low hepatic glucose production (Jenni et al, 2008), however it has not been fully characterised within CPET on starting blood glucose levels pre-exercise, where there is a ramped increase in metabolic stress and intensity. Nevertheless, the possible consequences these deviations in energy metabolism may have on physical performance during CPET, as these metabolic markers can be used to plot intensity thresholds for individualised exercise prescriptions (Albouaini et al, 2007).

As previously discussed, glycaemic responses to exercise are largely overseen by the relative intensity (Zaharieva & Riddell, 2017), therefore an abnormal glycaemia pre-exercise of the CPET may lead to inaccurate identification of a true submaximal and maximal output of an individual. From a clinical point of view, an awareness of the changes taking place during progressive exercise may inform the nutritional and medicinal preparatory routines healthcare professional staff could make to enable completion of the test with safe blood glucose concentrations. A standardisation in procedural aspects of gathering blood sample is key in attaining reproducible results for inter-study comparisons. Given the value of its incorporation as part of a screening procedure in clinical research trials, there is a need to detail the typical glycaemic responses to CPET using a standardised methodology that encourages both safe practices and allows for reproducibility in test-retest situations.

1.10. CPET protocols and guidelines

Within CPET the purpose of the test is to understand the functional capabilities of the individual and help govern the choice of protocol. When evaluating individuals, both a cycle ergometer and treadmill can be used, but with their advantages and disadvantages (Table 4). However, within the clinical sector the cycle ergometer is more likely to be used. In comparison to another protocol using a treadmill for CPET such as the Bruce protocol. This determines an estimated O_2 utilization from reporting metabolic equivalents (METs). However, this is determined by using linear-regression and has been shown to overestimate oxygen utilization and using a treadmill is common clinically to be associated with a degree of error (Myers, 1996). Another procedure call the ramp protocol uses an ergometer, measuring ventilatory inhaled gases provides a more accurate quantification of the VO_2-Work rate relationship (Porszasz, Stringer & Casaburi, 2007) and the individuals functional capacity, as this is vital for exercise prescriptions with people who have clinical comorbidities (Forman et al, 2010).

Variable	Cycle Ergometer	Treadmill
Peak Oxygen Content	Lower	Higher
Work Rate Measurement	Yes	No
Blood Gas Collection	Easier Application	Difficult
Noise and artefacts	Less	More
Safety	Safer	Less Safe
Weight bearing in obese Subjects	Less	More
More Appropriate for	Patients (clinical)	Active Healthy Subjects

Table 4. Comparison in the efficacy of an ergometer and treadmill in the application of CPET. From the American Thoracic Society (2003).

The workload is measured in Watts (W), using the more popular ramp slope method ($W.min^{-1}$), which is most popular methodology used (Whipp, Davis, Torres & Wasserman, 1981), which can be manipulated to increase the wattage every 10-60 seconds (Swain, Abernathy, Smith, Lee & Bunn, 1994). The advantages of this procedure show that (1) it evaluates the exercise response across the entire range of functional capacity; (2) the initial work rate is low and the duration of high intensity exercise is low; (3) the protocol is short of duration during the incremental phase; (4) it permits the assessment of normality of the exercise response; (5) it permits the identification of the cause of the exercise limitation; and (6) it provides an appropriate frame of reference for training or treatment targets (Levett et al, 2018).

Using an ergometer, optimal exercise duration is short and quantified around 8 to 17 minutes, which is dependent on the participant (Buchfuhrer et al, 1983). Typically, before the incremental wattage increase begins a 3 minute of resting data collection followed by 3 min with a free resistance or very

low resistance (10-20 Watts), and then followed by a uniform increase in work rate until the limit of tolerance is attained (incremental phase) (Levett et al, 2018). The ramp slope can be alternated from 10, 20, 25 W min^{-1}, however in some cases lower values can be used (5-15 $W.min^{-1}$), this is dependent on the individual's current fitness levels. Manipulating the protocol to the individual is of high importance, primarily because if the workload increase is too large (<6 min), this can lead to an early termination, therefore condensing the data and to cause a non-linear relationship between the VO_2 and work rate (Forman et al, 2010), which can reduce the reliability of the data interpretation (Levett et al, 2018). Therefore, pre-analysis of the individual's medical history and baseline characteristics (Height, age, weight, fitness levels etc.) are of high importance when applying the correct workload dosage and to identify any potential contradictions to exercise testing (Myers et al, 2009). Post-exercise, it has been advised for a short recovery phase (3-5 min) of cycling to prevent venous pooling in the legs and monitoring of the subject should continue until physiological markers are reverting to baseline.

1.11. Ventilatory Markers in CPET.

CPET provides a large assessment of the responses of the pulmonary, cardiovascular, metabolic, and haematological systems. The main incorporation in CPET is respired gas analysis using ventilatory flow measurements permitting the calculation of O_2 uptake ($\dot{V}O_2$), CO_2 ($\dot{V}CO_2$), and ventilation (VE) by breath-by-breath analysis. Oxygen uptake (VO_2) is determined by cellular demand up to the level that equates to maximal rate of O_2 transport and delivery. $\dot{V}O_2$ max is the metabolic rate which is defined as the maximal point of oxygen uptake achieved during a rapid incremental test until reaching a physiological end point (Hill & Lupton, 1923). This is underpinned by the Fick principle, which is the total oxygen consumption (VO_2) in the tissue must equal the delivered oxygen less the returned oxygen. The delivered oxygen is equal to the cardiac output (CO) multiplied by arterial oxygen content (C_aO_2) and returned oxygen is equal to cardiac output multiplied by the venous oxygen content (CvO_2), creating $\dot{V}O_2 = CO \times [CaO_2 - CvO_2]$ (Wasserman, Hansen, Sue, Stringer & Whipp, 2005) which reflects the maximum ability to transport and use O_2.

Rigorous determination of $\dot{V}O_{2max}$ relies on the demonstration of a plateau in $\dot{V}O_2$ during the incremental work rate, as this reflects the point where anaerobic by-products start to accumulate due to aerobically produced CO_2 in the muscles, causing a non-proportional increase in VO_2, therefore reducing muscle mechanical efficiency (Zoladz et al, 1998). $\dot{V}O_2$ max is the attainment of a physiological limitation at one or more points in the O_2 transport pathway between the lungs and the site of mitochondrial O_2 consumption at the cytochrome terminus of the electron transport chain (Wagner, 2000). Therefore, any atypical response of the convective respiratory or cardiovascular O_2 fluxes will result in an abnormally lower $\dot{V}$O2max (Levett et al, 2018). This combination of VO_2 versus work rate in CPET has traditionally been used as the best evidence of $\dot{V}O_2$max (Albouaini, Egred, Alahmar & Wright, 2007). Evidence shows that T1DM versus health adults can elicit a faster leg muscle deoxygenation suggesting

limited O_2 delivery is plausible for earlier fatigue and reduce the time to anaerobic threshold (Peltonen, 2012).

1.12. Anaerobic Threshold during CPET

The anaerobic threshold provides an understanding of submaximal exercise rate and defined as the $\dot{V}O_2$ above which arterial (lactate) first begins to increase systematically during an incremental exercise test and reflects an inadequate supply of O_2 supply to the mitochondria and increased glycolysis (Levett et al, 2018). Thus, the anaerobic threshold (AT) has shown to be an important assessment tool to assess the ability of the cardiovascular system to supply O_2 at a certain rate to prevent muscle anaerobiosis during exercise testing (Wasserman, Beaver & Whipp, 1990). Furthermore, it can help predict comorbidities and complications in a range of populations with a high degree of accuracy within CPET (Moran, Wilson, Guinan, McCormick, Hussey & Moriarty, 2016).

There have been many studies which try to understand the efficacy of using different physiological markers and methodologies in determining the anaerobic threshold (Cheng et al, 1992, Dickstein et al, 1990, Caiozzo et al,1982, Beaver et al, 1985), which in some cases have demonstrated a wide variation between methods (Vainshelboim et al, 2017). However, some studies have demonstrated the use of a combination of physiological markers and methodologies to determine the anaerobic threshold and has shown to have accuracy of AT determination (Gaskill et al, 2001, Levett et al, 2018). Within multiple studies the use of gaseous exchange data has been used to determine the AT using the V-slope method, this uses regression analysis of the slopes of the CO_2 (VCO_2) versus O_2 (VO_2) uptake plot. This method has shown to be useful within CPET due to its physiological application in understanding cardiac and pulmonary comorbidities (Wasserman et al, 1994, Wasserman, Beaver & Whipp, 1990). This is due the relative increase in lactate concentrations from an incremental workload until a distinct metabolic rate is reached where lactate starts to steepen linearly. This steepening is produced by an increase CO_2 production from the extracellular bicarbonate HCO_3 buffering, which causes an increased alveolar CO_2 output relative to O_2 uptake therefore causing an inflection point in VCO_2 indicative of accelerated glycolysis and lactic acidosis (Wasserman et al, 1994, Beaver, Wasserman, Whipp, 1986, Beaver, Wasserman, Whipp, 1990).

In addition, ventilatory efficiency can be assessed by the evaluation of the rise in minute ventilation ($\dot{V}_E$) and end tidal PO_2/PCO_2 which helps reflect the amount of oxygen or carbon dioxide gas dissolved in the blood, showing the effectiveness of the lung pulling oxygen into the blood stream from the atmosphere vice versa. This helps us to determine AT, which is identifying hyperventilation relative to oxygen. At the AT the excess $\dot{V}CO_2$ that is generated from anaerobic glycolysis results in a proportional increase in $\dot{V}_E$ as this is driven by $\dot{V}CO_2$ and starts to further increase with respect to $\dot{V}O_2$, this is

reflected in the end-tidal oxygen tension, which is a reflection alveolar oxygen tension and refers to the amount of oxygen at the end of exhalation ($PETO_2$) and can be assessed in $\dot{V}E/\dot{V}O_2$ (Levett et al, 2018). This reciprocates a decrease in $PETCO_2$, which is the end-tidal oxygen tension and the amount of carbon dioxide left in the alveoli at the end of exhalation, this is due to the respiratory compensation for metabolic acidosis.

Studies have reviewed the efficacy in the methodology of the lactate threshold, which is measured directly from lactate concentration with a good detection from a log-log transformation of lactate and work rate or $\dot{V}O_2$ (Beaver, Wasserman, Whipp, 1985) and developments in new software (Newell et al, 2007) using algorithms to identify significant points of change also using log-log transformation. Furthermore, studies have shown combining multiple methods of determining the lactate threshold using lactate markers has shown similarity and reproducibility (Davis et al, 2007). This threshold helps find the deflection point at which the Lactate/Pyruvate ratio increases, as bicarbonate changes with lactate (Wasserman, 1987).

Within various studies, ventilatory threshold methodology, such as the V-slope method, has been shown to present highly reproducible and accurate data in healthy and patients with certain metabolic comorbidities (Wasserman et al, 1994, Sue, Wasserman, Moricca & Casaburi, 1988). Furthermore, due to the reliability of ventilatory threshold methodology (V-Slope), the non-invasive nature of applying this is a lot more preferred than using invasive arterial lactate analysis (Wasserman, Whipp, Koyl, Beaver, 1973).

9.3. Cardiovascular Markers in CPET.

When conducting CPET cardiovascular parameters should be monitored throughout the test, for safety of the individual. This can include heart rate, blood pressure, and an electrocardiogram (ECG) recording system for continuous monitoring of heart rate and rhythm to see if there are ischemic electrocardiographic changes during exercise and recovery. CPET in evaluating atypical responses has been demonstrated that it represents a solid understanding of cardiovascular complications through graded exercise to VO_2 peak and is seen as the gold standard in assessing cardiac functional limitations (Mancini et al, 1991, Beniaminovitz & Mancini, 1999, Guazzi et al, 2012). Furthermore, it has become of high relevance in assessing individuals with T1DM due to the increased need for rehabilitation programs and guidance in assessing cardiac variability in CPET for people with diabetes (Buckley et al, 2020).

During the start of the CPET the immediate response of the cardiovascular system to exercise causes and increase heart rate due a decreased vagal tone and an increased sympathetic outflow and heart rate will increase linearly with work rate and VO_2. However, this interaction for optimal O_2 delivery depends on other external interactions between the functional components of the O_2 transport chain, which entails oxygenation of the blood in the lungs, normal O_2 transport capacity of the blood by cardiac output (CO) (Guazzi et al, 2017). Therefore, any small changes in this sequence to provide O_2 to the working muscles can produce lower optimal outputs in performance, and these changes in heart rate variability can be assessed when using CPET and cardiovascular markers.

The major pathophysiological reason for a low $\dot{V}O_2$ peak in individuals can be described by the Fick principle ($\dot{V}O_2 = CO \times [CaO_2 – CvO_2]$. Based on this understanding of delivery and extraction are the two-underpinning processes, which convey O_2 use through the cellular pathways (Balady et al, 2010). This reflects on blood oxygen saturation per heartbeat, (oxygen pulse, OP). During CPET the relative contribution of stroke volume to cardiac output is dominant during the initial and transitional phases of the exercise. With an accelerated increase in OP in the initial stages of exercise, however near peak slows near termination; this plateauing effect reflects the limitations in vascular perfusion and cardiac performance (Mezzani, 2017). Thus, the use of CPET is important in our understanding that might aid evaluation of exercise tolerance in the cardiovascular system and its limitations in people with type 1 diabetes.

1.13. CPET in Type 1 Diabetes

For people with type 1 diabetes, CPET can be employed to understand the physiological responses to the exercise and underpin any markers in which may have impact on performance (Baldi et al, 2010; Turinese et al, 2017). Indeed, recently efforts have been made in translating into clinical practice and provide recommendations for correct protocol and safety (Buckley et al, 2020; Kosinski, Besson & Amati, 2019).

The determination of exercise and aerobic rate ($\dot{V}O_2$ max) is of special interest with populations with T1DM, as it had been demonstrated that using CPET is strong predictor of physiological complications (Gusso et al, 2008, Moser et al, 2017). However, the influence of T1DM is complex; several studies have shown a reduced aerobic capacity and shorter time to fatigue when compared to their non-diabetic counterparts of the same anthropometric conditions (Komatsu et al, 2005; Faulkner, Quinn, Rimmer & Rich, 2005; Nadeau et al, 2010; Moser et al, 2017), nonetheless individuals with T1DM can still show similar response in $\dot{V}O_2$ max to healthy individuals (Baldi et al, 2010, Niranjan et al, 1997) which was shown to be dependent of on tight glycaemic control. Multiple mechanisms behinds exercise capacity ($\dot{V}O_2$ peak) has been evaluated in T1DM which have established a lower ventilation ($\dot{V}E$) at peak exercise (Komatsu et al, 2005), a lower lung diffusion capacity (Peltonen et al, 2012; Strojek, Ziora, Sroczynski & Oklek, 1992). Furthermore, there has been research showing a reduction in cardiovascular ability with a restriction in cardiac output and stroke volume (Gusso et al, 2012; Hägglund et al, 2012) and evidence to suggest resting heart rate variability pre-exercise in CPET in the prediction of lower $\dot{V}O_2$ max (Wilson et al, 2017). This establishes the impairments in O_2 delivery and extraction to prolong exercise performance duration and intensity in T1DM. However, most studies reported the attainment in performance is due to the implications of HbA1c levels (Moser et al, 2018; Wheatley et al, 2011; Baldi & Hofman, 2010). However, a recent study suggests no relation in HbA1c levels during CPET but a deviation in heart rate dynamics, which altered performance in T1DM versus the non-diabetes counterparts (Eckstein et al, 2021).

Despite this variation in understanding physiological responses during CPET in T1DM, this may be due to the differences in CPET protocol, and the type of individual as each person has a differentiation in T1DM. Furthermore, there is a gap in research where there are limited studies in assessing blood glucose responses continuously throughout CPET, which could help underly metabolic responses and cardiopulmonary limitations during CPET.

1.14. Statement of the problem

It is clear there is limited well-controlled research detailing the glycaemic responses during CPET and the impact glycaemia has during testing in individuals with T1DM. It is important to understand the glycaemic variability and reproducibility within a CPET test as the resultant data is of clinical relevance. Thus, the aim of this study is to develop further knowledge on glycaemia patterns to the performance of CPET and detail the impact of performing the CPET under different glycaemic states (eu- or hyper-glycaemia) in individuals with T1DM. Such data has the capacity to optimise performance within testing and fully glean insight into cardio-respiratory performance.

1.15. Purpose of the study

The main aim of this thesis was to -

1. Characterise blood glucose responses in CPET in T1DM.

2. Assess the impact of hyperglycaemia vs euglycemia on physiological and metabolic responses during CPET in T1DM.

1.16. Objectives

Aim 1: Characterise blood glucose responses in CPET in T1DM.

- To assess the blood glucose response from baseline pre-exercise throughout CPET.
- To assess the change in blood glucose responses from a progressive CPET intensity.
- To assess the rate of the change in blood glucose responses from a progressive CPET intensity.

Aim 2: Assess the impact of hyperglycaemia vs euglycemia on physiological and metabolic responses during CPET in T1DM.

- To understand the effect of starting glucose levels (Hypoglycaemia vs. Euglycemia) on physiological biomarkers and metabolic demand on CPET performance.

Chapter 2 – Methodology

Chapter 2 – Methodology and materials

2.1. Study design

This study was conducted according to the Declaration of Helsinki, and the National Research Ethics Committees of the United Kingdom and Austria approved all procedures. All participants were provided with a full written and verbal description of the study and gave informed consent prior to taking part. This study was a pooled analysis of data collated from three randomised controlled trials (German Clinical Trials Register: DRKS00013509. Clinical trials.gov: NCT02075567, NCT01704417) that employed identical, equipment and standardised cardiopulmonary exercise test protocols. Cardiopulmonary and metabolic data were transferred into a database for investigation. All patient-specific information anonymised via the use of non-identifiable participant codes.

2.1.2. Inclusion and exclusion criteria

The inclusion criteria were diagnosis of T1D for ≥12 months; age 18 to 65 years (both inclusive); body mass index of 18.0 to 29.4 $kg.m^2$; use of multiple daily injections of insulin for ≥12 months; body mass-specific peak oxygen uptake of ≥20 $mL.kg^{-1}.min^{-1}$, and a status of being physically active as assessed by the International Physical Activity Questionnaire (IPAQ) Short Form. Use of contraception for female participants needed to be enquired. Main exclusion criteria were the presence of a diabetes related disease (i.e., proliferative retinopathy or maculopathy, severe neuropathy), recurrent severe hypoglycaemia (>1 severe hypoglycaemic event during the previous 12 months), hypoglycaemia unawareness, hospitalization for diabetic ketoacidosis during the previous 6 months or any other condition that would interfere with trial participation/evaluation of results.

2.1.3. Participant characteristics

The baseline characteristics of the thirty-six individuals with T1D included in this study are displayed in Table 5.

Characteristics	Mean±SD	Range (min-max)
Female (n; %)	7 (22)	-
Male (n; %)	29 (78)	-
Age (years)	32±13	18 - 65
Height (m)	1.7±0.1	1.6 – 1.9
Weight (kg)	77.2±9.1	58.9 – 96.9
Body mass index (kg.m²)	25.4±3.2	20.0 – 32.2
Duration of diabetes (years)	17±10	2 - 42
Age of diabetes onset (years)	15±11	1 - 48
HbA1c (%)	7.3±1.1	5.0 – 9.5
HbA1c (mmol/mol)	56.0±11.5	31.0 – 80.0
TDD (IU.kg.bm^{-1})	0.58±0.24	0.20 – 1.40

Table 5. Baseline characteristics of the study participants. Data are presented as mean±SD. N: number of participants. M; Male. F; Female. TDD; Total Daily Insulin Dose (inclusive basal and bolus amounts).

2.1.4. Preliminary guidelines prior to laboratory attendance

Before laboratory attendance, participants were asked to avoid caffeine for 12 hours, and alcohol and physical exercise for 24 hours. Participants were told to be vigilant in the avoidance of hypoglycaemia (defined as a capillary BG value of ≤3.9 mmol.L^{-1}) by frequent monitoring for 24 hours. Whilst participants were instructed to maintain their routine dose of basal insulin, participants were advised to consume a meal alongside a 10-25% reduced dose of individualised bolus insulin 1-2 hours prior to arriving at the laboratory.

Arriving to the laboratory, participants were screened by a physician. Following successful inclusion against the study protocol criteria, participants provided a resting baseline capillary BG measurement. If pre-exercise BG was <6 mmol.L^{-1} the CPET was delayed, and participants consumed 10g carbohydrate ([CHO] Glucogel®, BBI Healthcare Ltd). BG was monitored every 10 minutes with further bolus CHO given as necessary where the type, time and amount were recorded until BG was above a target threshold (≥6mmol.L^{-1}). Thereafter, anthropometric, cardiovascular and diabetes data were collected.

2.2. CPET protocol

Participants performed a CPET on a workload-controlled cycle ergometer (Corival Recumbent, Lode, NL). The automated protocol integrated with cardiopulmonary equipment consisted of a 3-minute passive resting phase sitting on the bike (0 Watts), prior to a standardised 3-minute warm-up phase (20 watts), this was then followed by ramped 1-minute increases in workload until volitional exhaustion. To ensure a reasonably similar exercise duration between participants and the correct exercise intensity was applied the intra-test protocol increase in work rate ranged on their fitness levels from 10 (n=4), 15 (n=12) or 20 (n=20) watts, as assessed by an exercise physiologist against their IPAQ responses that categorised participants as low-, medium- or highly active. Total test duration was 25±3 minutes, and the time until the anaerobic threshold (AT) was 11±2 minutes and the time until the peak was 19±3 minutes. Incremental workloads increased by 17±3 watts per minute. A graphical overview of the CPET protocol used in this study is displayed in Figure 4.

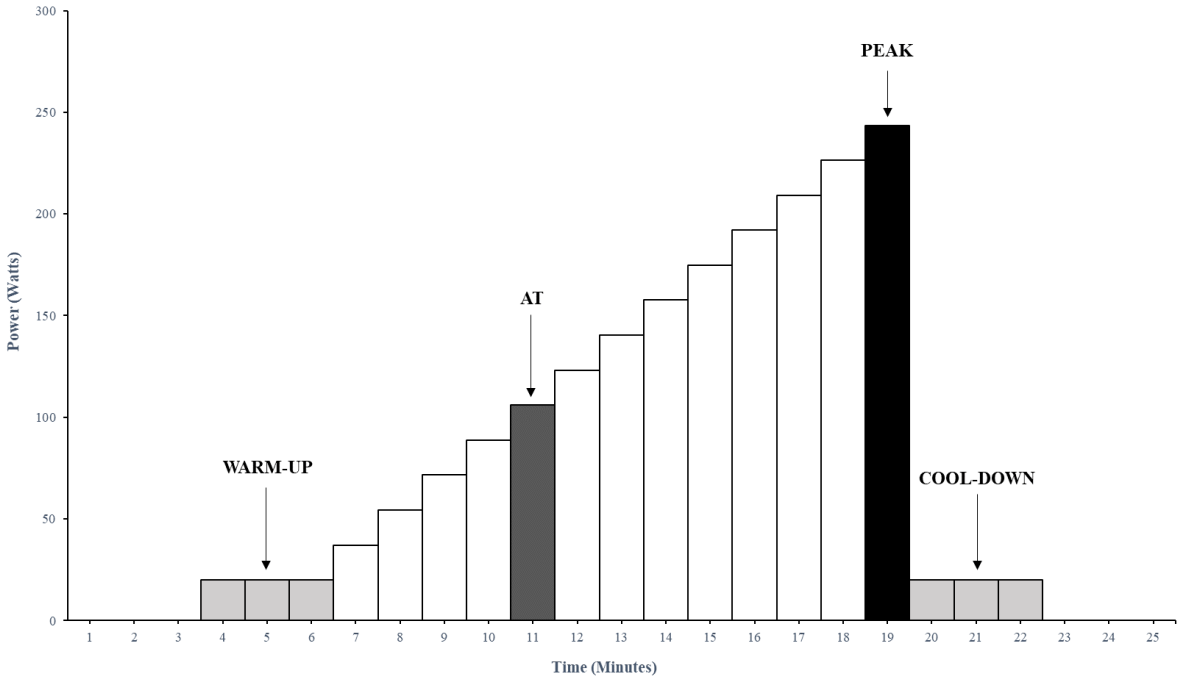

Figure 4. A graphical outline of the incremental workload protocol used for the CPET. The plot contains the mean workload increase data obtained as a part of this study.

The peak rate of oxygen consumption ($\dot{V}O_2$ peak) was defined as the highest O_2 uptake ($l.min^{-1}$ or $ml.kg^{-1}.min^{-1}$) obtained in the 30 seconds prior to test termination. $\dot{V}O_2$ peak was utilised over VO_2 max due to an inability to identify a plateau in some participants' O_2 consumption rates because of the short duration of exercise test and/or volitional termination occurring quickly upon achievement of peak power. All other cardio-respiratory and performance parameters were indexed to the $\dot{V}O_2$ peak value.

Volitional exhaustion occurred in all participants and defined by one or more of the following parameters, (i) an inability to maintain a pedalling cadence of >50 revolutions per minute (RPM) for >5 seconds, (ii) a respiratory exchange ratio (RER) of >1.1, (iii) a plateau in $\dot{V}O_2$. At the point of termination, the workload immediately decreased to 20 watts and participants were asked to continue pedalling through a 3-minute active recovery phase before completing a final 3-minute passive recovery period on the ergometer.

2.2.1. Identifying the Anaerobic Threshold

The anaerobic threshold (AT) was identified within CPET using various methods to provide accuracy and reliability. Firstly, the $\dot{V}CO_2$-$\dot{V}O_2$ relationship, with the modified V-slope index to identify the tangential breakpoint in the $\dot{V}CO_2$-$\dot{V}O_2$ relationship (Figure 5a) by using a linear line to visually identify the striation of linearity in VCO_2 and VO_2. Also, to identify the responses of ventilator equivalents for carbon dioxide (CO_2) and oxygen (O_2) i.e., $\dot{V}E/\dot{V}CO_2$, $\dot{V}E/\dot{V}O_2$ expressed as a function of $\dot{V}O_2$. This is identified when the $\dot{V}E/\dot{V}O_2$ relationship being flat starts to systematically increase while the $\dot{V}E/\dot{V}CO_2$ steadily decreases (Figure 5b). And the responses of the end-tidal PCO_2 and end-tidal PO_2 ($PETCO_2$ and $PETO_2$) expressed as a function of $\dot{V}O_2$ to understand the point of hyperventilation relative to $\dot{V}O_2$, where the $PETO_2$ starts to systematically increase and does not return to baseline (Figure 5c). Furthermore, the V-slope method (Figure 5d) using the $\dot{V}CO_2$-$\dot{V}O_2$ relationship which is split into linear regression lines below AT and above AT to identify the inflection point between the $\dot{V}CO_2$-$\dot{V}O_2$ relationship, the crossover of these segments being the AT point. Figure 5 provides a worked example of the methodologies used to determine the AT in this study. The AT was analysed using each of these methods by four independent exercise physiologists. The four individual sections of data analysed was compared to understand the coefficient of variations within each sample of the methods used. The V-slope methods using VO_2, and CO_2 were established as the significant methods used which had the lowest inter and intra-researcher irregularity, therefore this was the method used to determine AT.

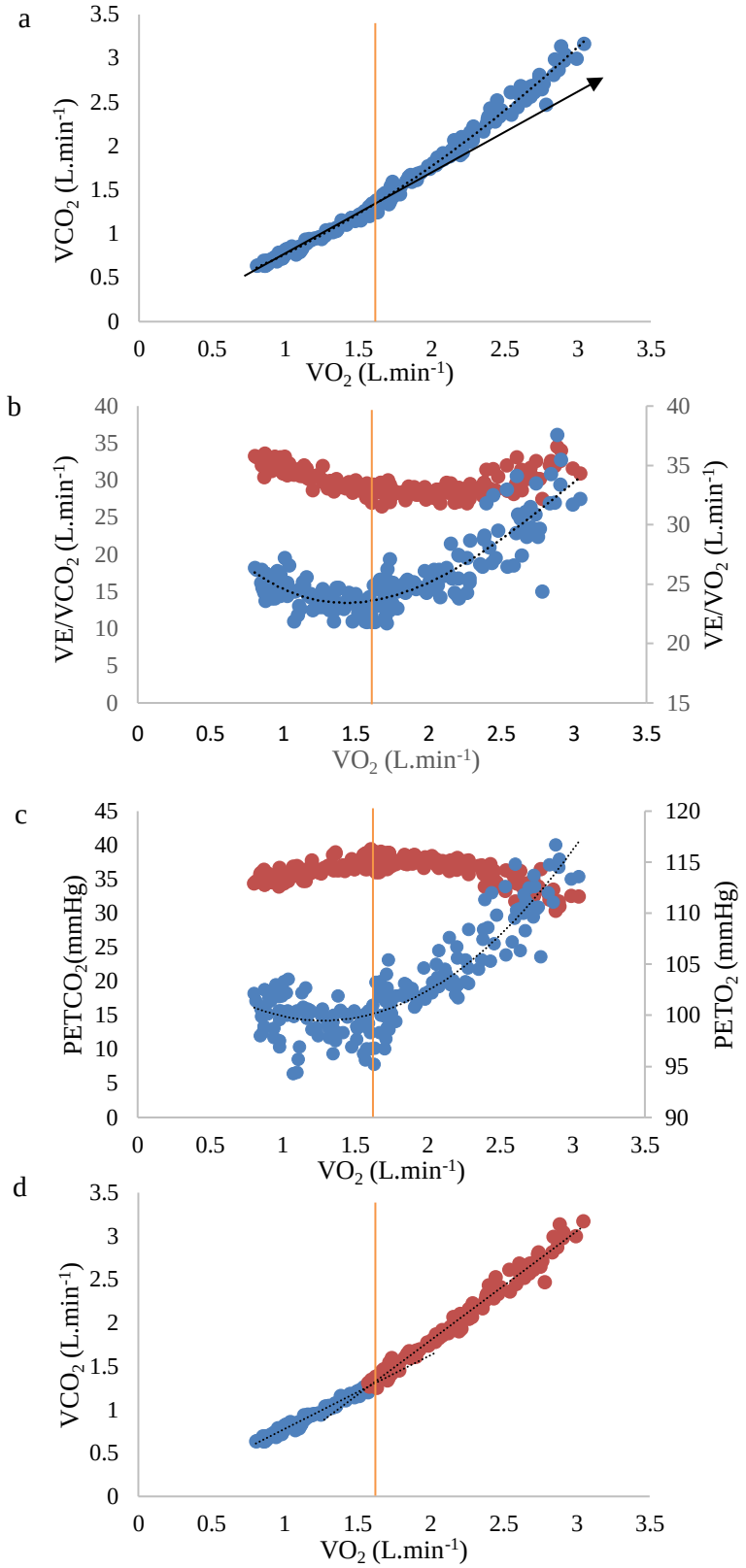

Figure 5. Example of the triad methodology used to determine the anaerobic threshold in this study (a) The V̇CO2–V̇O2 relationship, with the modified V-slope index of anaerobic threshold estimation. (b) The responses of the ventilatory equivalents for CO2 and O2 (V̇E/V̇CO2, V̇E/V̇O2) expressed as a function of V̇O2. (c) The responses of the end tidal PCO2 and end tidal PO2 (PETCO2 and PETO2) expressed as a function of V̇O2. (d) V-slope estimation, the V̇CO2-V̇O2 relationship is partitioned into linear Blue and Red regions within the region of interest demarcated by the orange vertical line. See text above for further details. The estimated anaerobic threshold is marked with the vertical orange line on all three plots.

2.2.2. CPET measurements and safety monitoring

Breath by breath data was measured using a pulmonary gas analyser (MetaMax® 3B; Cortex Biophysik GmbH, Germany). MetaSoft® software was used to help calibrate. If during calibration the recorded measurements were not identical to the standardized conditions (pressure, humidity, temperature) presented from the equipment, re-calibration would be permitted. Pressure was performed to measure the ambient temperature with the use of the digital barometer; the value would then be transferred into the software. The calibration of volume was performed by tightly connecting the gas sample line to the volume sensor and the calibration syringe, which corresponds with rest and maximum breathing volume (1 – 3 Litres). Performing volume calibration required the sliding of the syringe piston continuously between the stroke ends to produce minimum and maximum flow values. If these values were within the required ranges, then the data would be sent to the device. Gas calibration was completed using certified gases (Gas 1: Ambient Air, Gas 2: 15% O_2, 5% CO_2) with data displayed for standardised temperature and pressure for dry air.

All cardio-pulmonary data were subsequently averaged in 5 s intervals (Metasoft Software, Cortex Biophysik GmbH, Germany) for statistical processing and being transferred into a master file (Excel). Heart rate (HR) was recorded via chest belt telemetry (Polar Electro, Finland). Capillary blood samples (20 µl) were taken from the right earlobe (Accu-chek safe T pro plus lancets, Roche Diagnostics, Switzerland) to determine BG and lactate [BLa] at rest, for each minute during exercise, and at the end of both the active and passive recovery periods. Samples were placed in haemolysis solution and analysed within one hour using an enzymatic-amperometric analyser (Biosen C-line, EKF Diagnostics, Germany), the chemical titration of an electric current in which applies a difference between two opposing electrodes, therefore immobilising enzymes and detecting what is within the solution sample, does this. Calibration of the Biosen C-line was automatically calibrated (20 minutes) with prepared standards (STD), by starting calibration, start device from 'Stand-by' mode, then wait until the 'warm-start' is complete which is when the STD solution is applied to the machine at '1 STD'. Then place $ReadyCon_{pat}$ and $ReadyCon_{norm}$ (tubules for glucose and lactate) into C1 and C2. Once that is done the sensor test solution would be placed in probe position 1 and then place $EasyCon_{control}$ and $EasyCon_{pat}$ in probe positions 2 and 3. Finally, the calibration can be selected.

During CPET several measurements were made to assure participant safety during exercise (i) fingertip BG (0.4 µl) was taken every 4 minutes and analysed immediately via the inbuilt glucometer (Freestyle Libre glucometer, Abbott Inc), (ii) ratings of perceived exertion ([RPE], Borg scale11). A portable 3-lead ECG (eMotion Faros 180°, Bittium Biosignals Ltd., Finland) provided a wireless live stream of cardiac responses for safety.

2.2.3. Post CPET glycaemic monitoring

Following exercise, participants' BG concentrations were closely monitored. Where BG levels were <6 mmol.L^{-1} following CPET performance, a light meal without bolus-insulin injection was provided. Participants remained in the laboratory until BG reached ≥6.0 mmol.L^{-1} before departure.

2.3. Statistical analysis

All statistical analyses were carried out using IBM SPSS 26.0 (IBM Corp., Armonk, NY). A One-Way ANOVA and Bonferroni-corrected pairwise comparisons were used to examine time effects. To determine the influence of starting BG on performance parameters during CPET, participants were split into groups depending on whether they commenced exercise whilst euglycemic ([**Eu**] ≥4.0 - ≤10.0 mmol.L^{-1}) or hyperglycaemic ([**Hyper**] >10.00 mmol.L^{-1}). Intra-group differences were assessed by means of paired sample t-tests whilst inter-group differences were assessed via independent t-tests and inter group analysis was assessed using a 2-way-ANOVA. Forward stepwise linear regression was used to explore relationships between starting BG and metabolic and cardiovascular responses to CPET. $P<0.05$ were considered as statistically significant.

Chapter 3 – Results

3.1. Peak performance parameter outcomes

The peak physiological and metabolic responses to the CPET testing detailed in Table 4.

Parameter	Rest	Warm-up	AT	Peak	Active Recovery	Passive recovery
HR (bpm)	81±14	98±14*	130±15*	180±13*	133±12*	115±11*
Power (W)	0.0±0.0	20±0.0	114.5±35.7	242.8±62.3	20.0±0.0	0.0±0.0
Power ($W.kg.bm^{-1}$)	0.0±0.0	0.3±0.0	1.5±0.5	3.1±0.8	0.3±0.0	0.0±0.0
$\dot{V}O_2$ ($l.min^{-1}$)	0.4±0.1	0.9±0.3*	1.7±0.5*	3.2±0.9*	1.2±0.4*	0.6±0.2*
$\dot{V}O_2$ ($ml.kg\ bm^{-1}.min^{-1}$)	4.8±1.4	11.4±3.5*	22.4±6.5*	41.7±10.2*	16.1±3.8*	7.8±2.0*
$\dot{V}CO_2$ ($l.min^{-1}$)	0.3±0.1	0.7±0.2	1.6±0.5*	3.7±1.0*	1.5±0.4*	0.7±0.2
RER	0.8±0.1	0.8±0.6	0.9±0.1	1.2±0.1*	1.2±0.9*	1.1±0.1*
O_2 pulse ($ml.beat^{-1}$)	4.5±1.4	9.0±2.4*	13.5±4.0*	17.7±4.2*	8.0±1.9*	5.6±1.8
$\dot{V}_E$ ($l.min^{-1}$)	11.4±3.0	21.1±4.8*	40.0±10.4*	118.7±29.0*	44.0±10.4*	26.9±7.2*
$\dot{V}_T$ ($l.min^{-1}$)	0.8±0.3	1.1±0.3*	1.8±0.6*	2.5±0.6*	1.6±0.4*	1.1±0.3*
BF ($l.min^{-1}$)	15.9±4.4	20.3±5.1*	23.5±4.6*	48.7±11.5*	28.7±5.4*	24.6±4.9*
$\dot{V}E/\dot{V}O_2$ ($l.min^{-1}$)	26.1±5.2	22.3±4.3	22.2±3.6	35.7±5.9*	39.5±5.4*	40.5±6.6*
$\dot{V}E/\dot{V}CO_2$ ($l.min^{-1}$)	30.7±5.6	27.6±4.3	24.8±3.2*	31.2±5.6	33.1±4.1*	36.8±5.9*
$PETO_2$ (mmHg)	105.0±5.6	98.4±6.5*	99.5±6.3	118.4±5.4*	120.9±3.8*	121.2±4.5*
$PETCO_2$ (mmHg)	36.4±3.6	39.6±3.8	42.7±4.5*	33.3±4.7	32.0±3.3*	29.7±3.3*
BLa ($mmol.l^{-1}$)	0.8±0.3	0.9±0.3	2.0±0.7*	9.9±2.4*	10.9±2.6*	10.5±3.1*

Table 6. Peak physiological and metabolic responses to cardiopulmonary exercise testing (n=36, mean±SD). HR; heart rate. BLa; blood lactate. RER; respiratory exchange ratio. $\dot{V}O2$; volume of inhaled oxygen. $\dot{V}CO2$; volume of exhaled carbon dioxide. PETO2; Expiratory End-tidal CO2 Partial Pressure. PETCO2; Expiratory End-tidal O2 Partial Pressure. $\dot{V}E/\dot{V}O2$; Ventilatory Equivalent for O2. $\dot{V}E/\dot{V}CO2$; Ventilatory Equivalent for CO2. BF; Breathing frequency. VT; tidal volume. VE; minute ventilation. * indicates a significance in values from rest p=0.05.

3.2. Cardiopulmonary responses to CPET

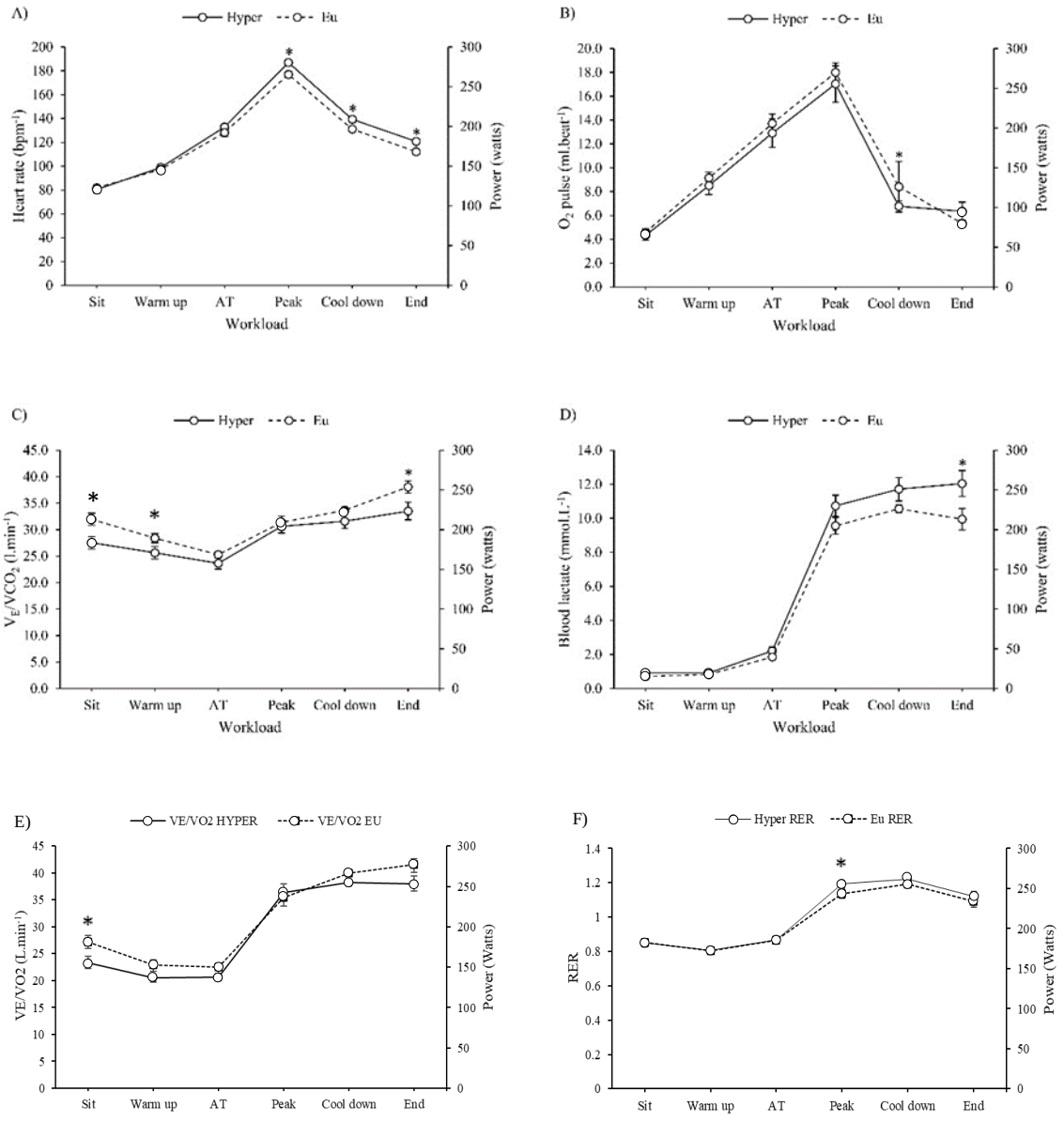

Figure 6. The influence of pre-exercise blood glucose concentrations on cardiovascular and metabolic responses to CPET in people with type 1 diabetes. * Denotes a significant difference of p≤0.05 between the hyperglycaemic and euglycemic groups for the identified value. A) denotes the heart rate response between Hyperglycaemic and euglycemic groups from pre-exercise phases, the anaerobic threshold, peak exercise, cold down and termination of the protocol. B) denotes the Oxygen saturation per heartbeat (O_2 pulse) response between Hyperglycaemic and euglycemic groups from pre-exercise phases, the anaerobic threshold, peak exercise, cold down and termination of the protocol. C and E) denotes the Ventilation of carbon dioxide and oxygen (V_E/VCO_2 & V_E/VO_2) between Hyperglycaemic and euglycemic groups from pre-exercise phases, the anaerobic threshold, peak exercise, cold down and termination of the protocol. D) denotes the blood lactate response between Hyperglycaemic and euglycemic groups from pre-exercise phases, the anaerobic threshold, peak exercise, cold down and termination of the protocol. F) denotes the respiratory exchange ratio (RER) between Hyperglycaemic and euglycemic groups from pre-exercise phases, the anaerobic threshold, peak exercise, cold down and termination of the protocol.

This data was formulated and analysed by taking the raw data from the 5 second by 5 second time points that was taken from each biomarker presented in figure 6 (from the MetaMax® 3B; Cortex Biophysik GmbH, Germany). To create minute by minute markers, a 30 second average was taken from start of each minute through the start of the minute, this is presented in section 5.1. in the appendices. Then formulating the points of interest (Sit, Warm-up, AT, Peak, Cool Dow, End) by averaging these time periods to get a singular numerical average for each point (see section 5.3.1).

Hyper presented with higher HR values at the peak workload (**Hyper**; 187±5 vs. **Eu**; 177±15 bpm, p=0.004) as well as during the active (**Hyper**; 139±8 vs. Eu; 131±13 bpm, p=0.035), and passive (**Hyper**; 121±8 vs. **Eu**; 112±11 bpm, p=0.031) recovery periods (Figure 6A). **Hyper** displayed lower O_2 pulse during the active recovery period (**Hyper**; 6.8±1.5 vs. **Eu**; 8.4±1.8 ml.beat^{-1}, p=0.015 [Figure 6B]) and higher BLa concentrations (**Hyper** 12.0±2.1 vs. **Eu** 10.0±3.2 mmol.L^{-1}, p=0.045 [Figure 6 D]) during the passive recovery phase. **Hyper** showed a lower VE/VCO_2 at baseline (**Hyper**; 27.4±3.6 vs. **Eu**; 32.0±5.8, p=0.021) and at warm-up (**Hyper**; 25.4±3.7 vs. **Eu**; 28.4±4.4, p=0.05) and at the passive phase of recovery (End) (**Hyper**; 33.5±5.3 vs. **Eu**; 38.1±5.8 L.min^{-1}, p=0.038, [Figure 6C]). **Hyper** also presented a lower starting VE/VO2 at sit (**Hyper**; 23.3±4.2 vs. **Eu**; 27.2±5.2, p=0.033, [Figure 6E]). Finally, **Hyper** showed a higher peak in RER (**Hyper**; 1.2±0.1 vs. **Eu**; 1.1±0.07, p=0.031, [Figure 6F]).

3.3. Blood lactate responses during CPET

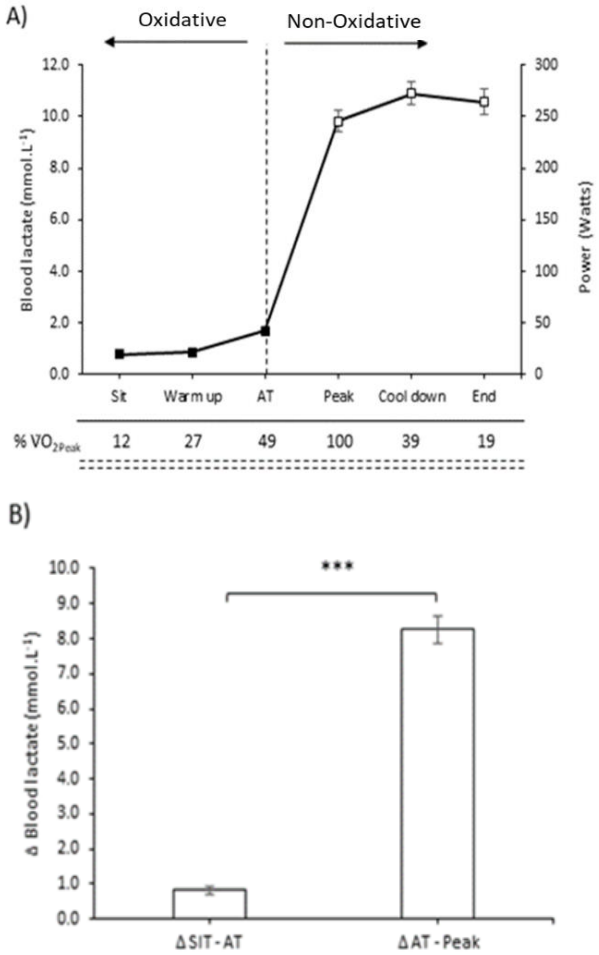

Figure 7. Figure 12 A: The response in blood lactate during CPET with reference to the power output at each point of interest. Figure 12 B The change in blood lactate below AT and above AT.

Figure 7A BLa rose significantly from sitting from the AT onwards (White icons; Figure A) and figure 7B The change in BLa from AT-Peak i.e. above the AT was significantly greater than the change in BLa from Warm Up-Anaerobic threshold (WU-AT) i.e. below the AT (Δ BLa below the AT; +0.90±0.69 vs. Δ Bla above the AT; +6.93±2.47 mmol.L-1, $p<0.001$)

Figure 8 represents the change in blood lactate and glucose over the period of the CPET shown as an expression under the percentage of heart rate max (%). This helps visualise the metabolic changes during a progression in physical stress and the increase in the magnitude in anaerobic lactate accumulation, and the stability presented in blood glucose despite this incremental increase in metabolic demand.

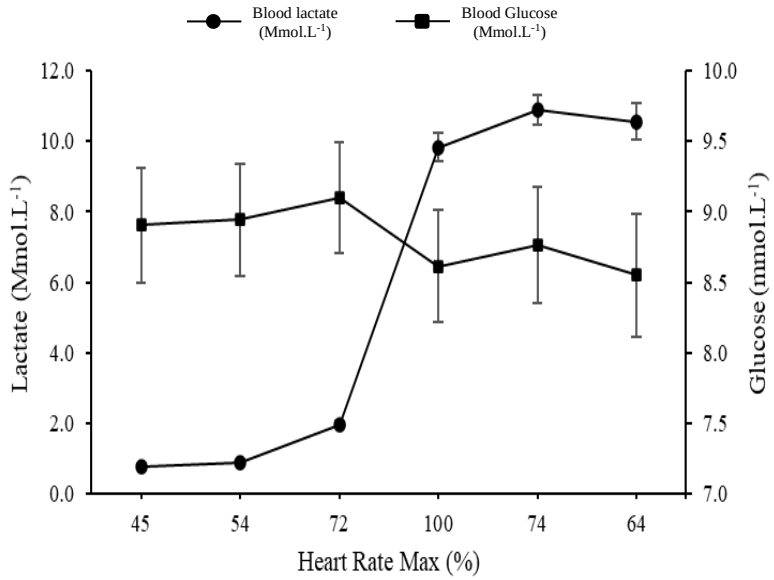

Figure 8. Represents the average change in lactate and glucose (mmol.L^{-1}) over CPET shown over the percentage of heart rate max (%) during and following exercise.

3.4. Blood glucose responses to CPET

Figure 9a represents each individual's blood glucose response pre-exercise, during anaerobic threshold, peak, 3 minutes active cool-down, and 3 minutes passive recovery. This shows the magnitude in the variation in blood glucose from each individual (9a) and the overall change from baseline to termination (9b) of the CPET. This demonstrates the characterisation of blood glucose and the individuality of adapting to the incremental physical stress during CPET.

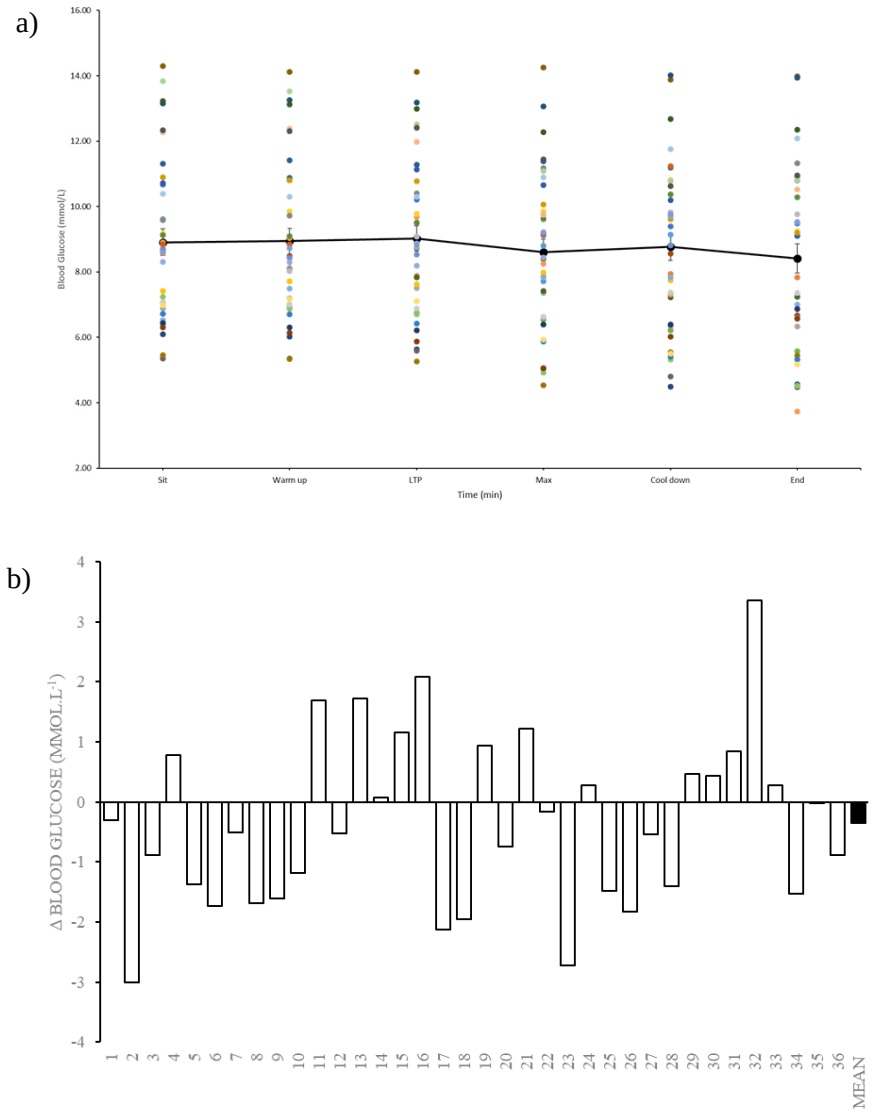

Figure 9 Represents individual blood glucose levels for each participant throughout CPET at specified points of interest. The black line characterises the mean with the standard error of the mean (SEM). 6b represents the overall change from the start of the CPET to the end of the trial. The black bar represents the overall mean.

BG responses to CPET were highly variable in people with T1D, with reductions of -3.01 $mmol.L^{-1}$ contrasted by increases of +3.36 $mmol.L^{-1}$. The mean change in BG over was -0.36 $mmol.L^{-1}$ (represented by the black bar). Linear regression revealed that starting BG was strongly and positively related to ending BG concentrations (β=0.849, $p<0.001$), but did not dictate the magnitude of change in BG over CPET (β=-0.179, $p=0.305$).

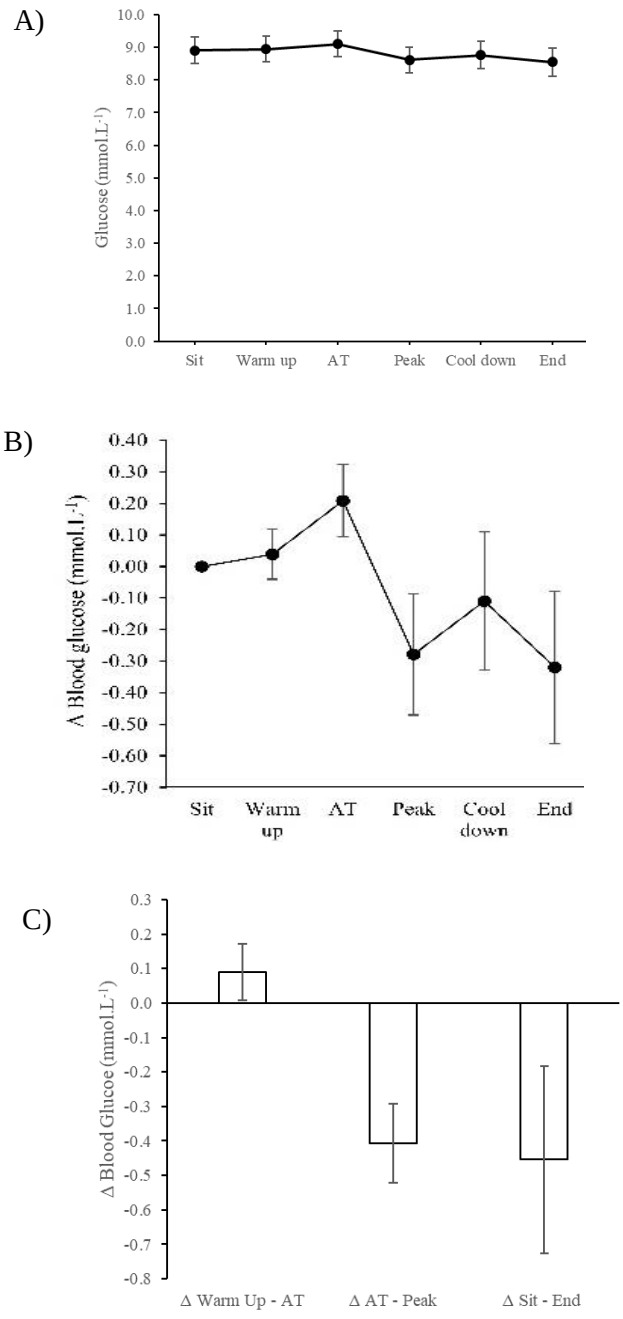

*Figure 10. Blood glucose responses to cardiopulmonary exercise testing when expressed as **A)** the absolute blood glucose concentrations at each work stage, **B)** the change in blood glucose from sit (in absolute units [mmol.L^{-1}]), and **C)** the change in blood glucose below or above the anaerobic threshold and over the entire CPET (Sit-End). AT; anaerobic threshold. Δ; change in blood glucose. *** denotes a significant difference of p<0.001 between values.*

BG values remained equivalent to sitting concentrations at each stage of the CPET protocol (Figures 10 A and B). The change in BG above the AT differed significantly from the change in BG below the AT (Sit–AT; +0.2±0.7 vs. AT-Peak; -0.5±0.8 mmol.L^{-1}, [i.e. Δ 0.7], p<0.001, Figure 10 C).

The rate of change was calculated by going through each participant one minute by one minute glucose data (please see section 5.2. in the appendices for a visual example). As we understood, we needed to find the change in blood glucose from its neighbouring minutely interval, once finding the change presented this would be divided by the time it took to get to this change. For the inactive periods this would be divided by three minutes, such as warm up, cool down and sit as this is the standardised time these periods took, and during exercise periods this would be by 1 minute. This would help us understand the change of blood glucose between these periods, but also help us understand the rate at this is changing to aid an explanation in why the appearance or disappearance of glucose is changing as such a different rate at certain stages of the exercise overall, or between individuals who are starting at a higher blood glucose level (hyperglycaemia) that individuals who aren't (euglycemia).

ROC values from the specified points of interest remained equivalent in comparison to values against baseline from Sit to Warm-up (Sit; 0.012±0.16 vs. Warm-up; 0.057±0.28 mmol.L^{-1}, p=0.41), AT (0.055±0.36 mmol.L^{-1}, p=0.51), Peak (0.097±0.42 mmol.L^{-1}, p=0.20). However, figure 11 shows the magnitude in the ROC between values showed that the change in the ROC from Sit-AT to AT-Peak was significantly different between groups (Sit-AT; Δ0.004±0.06 vs. AT-Peak; Δ-0.049±0.10 mmol.L^{-1}, p=0.01)

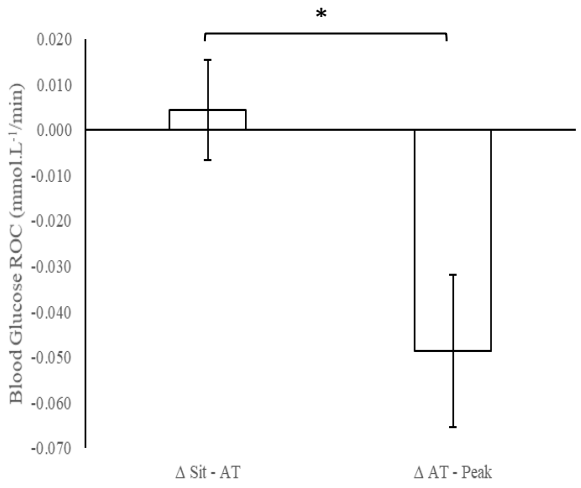

*Figure 11. The magnitude in the ROC of blood glucose from Sit-AT and AT-Peak. With Standard of the mean bars (SEM). * Denotes a significance between groups of <0.05.*

3.6. Euglycemic versus Hyperglycaemic starting blood glucose concentrations.

There were no inter-group differences in any anthropometric or diabetes characteristics data (Table 7 A]). Similarly, there were no differences between groups in the incremental workload increases or duration of CPET (Table 7 B]).

Parameter	Hyperglycemic (n=10)	Euglycemic (n=26)	P-value
A) Anthropometric and diabetes characteristics			
Gender (M; n [%] & F; n [%])	7 [70] & 3 [30]	21 [81] & 5 [19]	-
Age (years)	28±7	33±14	-
BMI ($kg.m^2$)	24.1±3.1	25.9±3.1	0.138
HbA1c (%)	7.4±0.8	7.2±1.1	0.664
Diabetes duration (years)	15.1±9.3	17.2±10.3	0.582
TDD ($IU.kg.bm^{-1}$)	0.5±0.2	0.6±0.3	0.461
B) CPET intensity and duration			
Workload (watt increase per increment)	18.0±3.5	16.9±3.5	0.413
Time to AT (minutes)	10.9±1.2	11.5±2.6	0.487
Time to Peak (minutes)	18.1±1.7	18.9±3.6	0.488
Time to End (minutes)	24.1±1.7	24.9±3.6	0.488
Time AT to Peak (minutes)	7.3±2.4	7.3±2.0	0.992

Table 7 Data to show the baseline anthropometric and diabetes characteristics A) and cardiopulmonary exercise testing intensity and duration B) of the study participants when stratified into groups depending on whether they commenced exercise whilst euglycemic (≥4.0 - ≤10.0 $mmol.L^{-1}$) or hyperglycaemic (>10.00 $mmol.L^{-1}$). Data presented as mean±SD. n; number of participants. M; Male. F; Female. HbA1c; glycated haemoglobin. TDD; Total daily insulin dose (inclusive of basal and bolus amounts).

3.7. Euglycemic versus Hyperglycaemic responses to CPET

Figure 12 displays the BG responses during CPET when data have been stratified into those who started with hyperglycaemic or euglycemic levels Figure 12A) the absolute BG concentrations at each work stage, Figure 12B) the change in BG from sit (in absolute units [mmol.L^{-1}]), and Figure 12C) the change in BG below or above the AT.

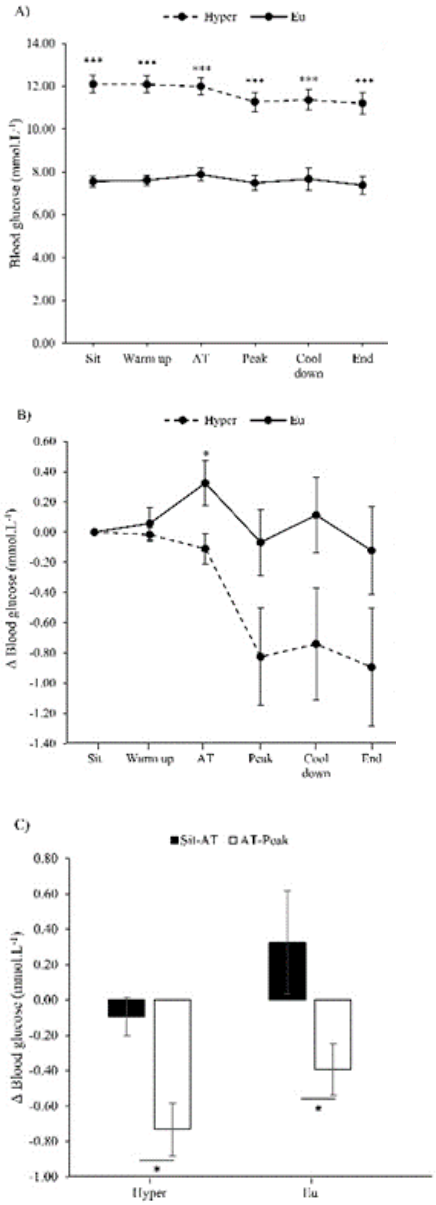

*Figure 12. Blood glucose responses during CPET when data have been stratified into those who started exercise hyperglycaemic or euglycemic and expressed as A) the absolute blood glucose concentrations at each stage, B) the change in blood glucose from sit (in absolute units [mmol.L^{-1}]), and C) the change in blood glucose below or above the anaerobic threshold. AT; anaerobic threshold. Hyper; hyperglycaemic. Eu; euglycemic. Δ; change in blood glucose. *** denotes a significant difference of p<0.001 between values. * Shows a significant difference of p≤0.05 between values.*

Inter-group comparison: We compared between the two hyperglycaemic and euglycemic groups was to see any significant changes between the points of interest and help us understand if there was anything in either group where there was any metabolic compensation from either the in the change of blood glucose when intensity increases from aerobic to anaerobic, and the magnitude in the change of the ROC between groups when starting at different blood glucose levels.

Hyper had higher BG concentrations throughout the entire CPET (all p<0.001 [*** icons Figure 12A]). At each workload stage, the BG in both groups remained comparable to their respective starting BG concentrations (solid versus straight line). The magnitude of change in BG from Sit-AT was significantly different between groups (**Hyper** Δ-0.09±0.35 vs. **Eu** Δ+0.32±0.76 p=0.030, [Figure 12B * icon at AT]). The delta in BG from sit to all other time points was comparable.

Intra-group comparison: By comparing between their own glycaemic groups were to see if there were any significant deviations or contributed to more of a stable blood glucose change or any points where there was significant rate in change of glucose turnover, which allowed to assess if there was any signific metabolic mechanism for the starting blood glucose levels. Therefore, allowing us to characterise blood glucose changes during CPET at a different angle.

In both groups, the change in BG from sit to AT (black bars) was significantly different to the change in BG from AT to peak (clear bars) (Figure 12C). **Hyper** Δ0.64±0.82 mmol.L^{-1}, p=0.035. **Eu** Δ0.72±1.0 mmol.L^{-1}, p=0.001. **Hyper** (black bars) from Sit-AT was had a significantly different magnitude in the ROC to AT-Peak (-0.04±-0.06 mmol.L^{-1}/min, p=0.032), however **Eu** (white bars) demonstrated that the magnitude in the ROC from Sit-AT to AT-Peak was not significantly different (0.01±0.08 mmol.L^{-1}/min, p=0.235, [Figure 12C]).

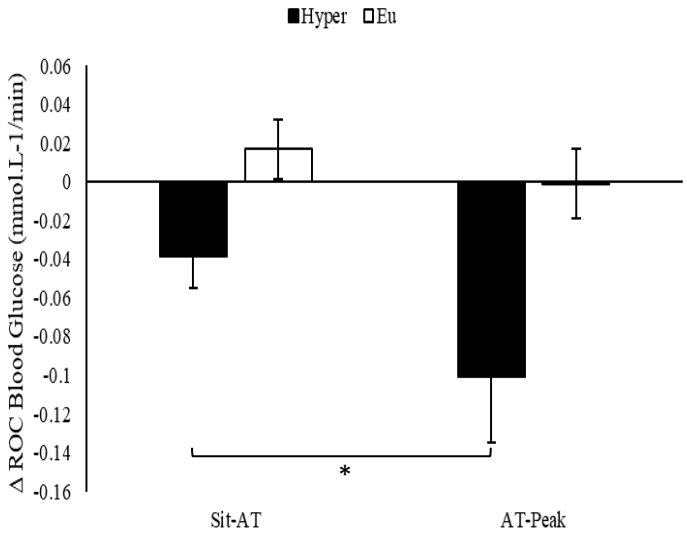

*Figure 13. The ROC of blood glucose when stratified into groups of individuals who started hyperglycaemic and who started euglycemic. Segmented into the magnitude of the ROC at baseline (Sit) to the anaerobic threshold (AT) in both groups. Hyper; Hyperglycaemic. Eu; Euglycemic. Δ; Change in blood glucose. Bars shown as Standard deviation of the mean (SEM). * Demonstrates a significance of p=≤0.05 between values.*

Between groups the magnitude in the ROC from Sit-AT was significantly different as **Hyper** showed a larger degree in the reduction of ROC (**Hyper**; -0.04±0.06 vs. **Eu**; 0.02±0.08 mmol.L^{-1}, p=0.022) and from AT-Peak (**Hyper**; Δ-0.10±0.11 vs. **Eu**; Δ-0.001±0.09 mmol.L^{-1}, p=0.021). However there was no difference in the ROC from baseline to termination (End) of the CPET (**Hyper**; -0.05±0.06 vs. **Eu**; -0.01±0.06 mmol.L^{-1}, p=0.071) (Figure 13).

Chapter 4 - Discussion

4.1 Summary of main aims

The aims of this thesis were to:

Aim 1: Characterise blood glucose responses in CPET in type 1 diabetes.

Aim 2: Assess the impact of glycaemia (hyperglycaemia and euglycemia) on physiological and metabolic responses during CPET in T1DM.

4.2. Cardiopulmonary Markers within CPET protocol.

Within our cohort of participants with type 1 diabetes, we have characterised the physiological and metabolic response to a well-controlled CPET from low intensity to exhaustion. Further, we have stratified individuals based on their starting blood glucose. We were able to demonstrate the physiological stress in a progressive manner within CPET to underpin each physiological biomarker during exercise.

CPET demonstrated the significant increase in cardiovascular demand to exhaustion, in this process of increase intensity and duration the delivery in O_2 to the mitochondria is essential for aerobic capacity (Wasserman, 1994, Wasserman, 2000) which creates this linear relationship with a progression in work rate (Milani, Lavie, Mehra & Ventura, 2006). O_2 Pulse showed a significant difference from rest during rest to peak within CPET, this in hand shows the saturation of oxygen consumed per beat ($CO \times [CaO_2 - CvO_2]$) and can help understand the peripheral O_2 extraction along with the linearity of VO_2/VCO_2 relationship during exercise. Our study shows that the reduced affinity for oxygen during CPET can help reflect the limitation in oxygen extraction and utilization within individuals with metabolic dysfunction in T1DM (Mezzani et al, 2017). The increase in oxygen uptake must be balanced is to increase cardiac output (Heart Rate x Stroke Volume) with an increased blood flow to the lung occurs in CPET, by the increase the cardiac output and vasodilation of pulmonary vessels optimum O_2 extraction can occur (Balady et al, 2010). If one of these cardiopulmonary markers are atypical in the response to the CPET, a reduction in exercise performance can occur, which is why it is of relevance to collect these markers to understand these responses in T1DM.

Ventilatory markers showed significant increases during the incremental exercise a point of interest at a majority from warm up to peak. During a progressive increase in intensity and duration in CPET, VE responses are tightly regulated by blood flow (cardiac output) and the development of lactic acidosis and proton (H^+) production (Guazzi et al, 2017) from the prevailing anaerobic metabolism due the imbalance of O_2 supply to the working skeletal muscles, this in hand further increases VE/VCO_2 release and the consequent amount of VE until maximal exhaustion (Balady et al, 2010). By the increase in these ventilatory markers during CPET allows us to use anaerobic threshold methodology and find the point of the change into anaerobic pathways, which in hand can help us distinguish the effects this has on other cardiopulmonary markers.

During exercise demonstrated a change in the shift in metabolic fuel choice, as RER increased significantly after the point of the anaerobic threshold (>1.1 RER) which implies significant anaerobic metabolism above the anaerobic threshold during progressive intensity (Mezzani et al, 2017). This is greatly influenced by substrate utilization and within T1DM it has been shown that CHO utilization can be impaired, T1DM subjects may use a larger amount of lipid substrates (Turinese et al, 2017). Therefore, understanding the use of substrates for fuel during progressive high intensity periods in T1DM can help understand exercise tolerance and glycaemia levels. As we can see during CPET the progressive increase in intensity and moving into the non-oxidative pathways as lactate significantly increasing above rest (Mezzani et al, 2009), this in hand causes buffering period which delay the point that the blood lactate level rises to a level where we are forced to slow down, this in hand yields an excess amount of carbon dioxide to make the $\dot{V}O_2/\dot{V}CO_2$ become steeper during exercise (Beaver, Wasserman, Whip, 1985). This ramped increase in intensity reaches a point where intracellular bicarbonates can no longer stabilize the exercise-induced metabolic acidosis (70-80% $\dot{V}O_2$ max) (Sietsema, Stringer, Sue & Ward, 2020). Furthermore, as it has been shown T1DM individuals can elicit and lower anaerobic threshold during CPET in comparison to health individuals (Komatsu, Neto, Chacra & Dib, 2010), this is resultant due to factors such as an increase in blood glucose levels and a low lactic acid clearance during exercise, furthermore due to a reduction in O_2 from a reduction in ventilatory capacity uptake (Komatsu, Neto, Chacra & Dib, 2010). This understanding can help evaluate fitness tolerances and for premature anaerobic glycolysis in T1DM.

4.3. Blood glucose concentrations during CPET

Throughout the CPET duration it was found there was no significant change in blood glucose concentrations at the points of interest. Literature has demonstrated throughout intensities of modest exercise and longer duration elicits a decrease in blood glucose over the exercise period (Clarke et al, 1997, Campbell et al, 2013) as the glucose disappearance can increase 2-3-fold due to increase muscular skeletal uptake (Wasserman & Cherrington, 1991) and the inability for hepatic glucose production to maintain glycaemia from existing glycogen stores, which can lead to subsequent hypoglycaemia (Riddel et al, 2013). Furthermore, in comparison to high intensity, combining short bursts of power in a short duration has thoroughly been shown to increase blood glucose appearance (Fahey et al, 2012, Yardley & Sigal, 2015) due to the rise in catecholamines, cortisol, and growth hormone levels leading the increase of hepatic glucose production and inhibition of insulin-mediated fall in glycaemia (Marliss, Vranic, 2002). If pre-exercise factors are not corrected hyperglycaemia can pursue (Aronson, Brown & Riddell, 2019). Therefore, we suggest the reason why our cohort didn't result in the change in blood glucose demonstrates the combination of these two intensities and the duration of the CPET can elicit an increase intensity and physiological stressors that can cause a change in glucoregulatory responses

in T1DM individuals, which characterised a stability in glucose response in CPET due to the increase in high intensity, which produced an increase in glucose appearance. These findings supported and went in line with Moser *et al*, (2017) who reported no significant differences between pre (9.3±3.4 mmol.L^{-1}) and post exercise (10±3.2 mmol.L^{-1} p=0.06) with similar CPET procedure with a 1 minute progression increase in intensity (30W for females, 40W for males) and implementing pre-exercise blood glucose levels by applying carbohydrate consumption in individuals with low pre-exercise glucose levels (4.4 mmol.L^{-1}) or small bolus adjustments if glucose levels are too high for exercise (13.9 mmol.L^{-1}). This stability in the glycaemia was still achieved with different insulin adjustments to ours. A study by Adolfsson et al, (2012) supported our study and helped characterise the variation in glycaemia during a CPET in women (n=6) and men (n=6), by using a similar ramp protocol (15W for women, 25W for men every 30 seconds) until volitional exhaustion but starting the participants at 100W (women) and 125W (men). Insulin regimes were kept the same, however adjusted to the individual. This found that from pre-exercise to maximal, blood glucose dropped −1.5 ± 1.5 mmol.L^{-1} compared to non-diabetics of +0.03 ± 0.9 mmol.L^{-1}, which was seen as significant but showed similar responses, respectively. The drop in blood glucose within the diabetic group helps characterise the effect of CPET protocol around glucose variability when properly applied, however, the limitation of this study would be the population size as this would help increase the level of confidence and remove any degree of error.

The overall magnitude of blood glucose between baseline to the anaerobic threshold (+0.2±0.7 mmol.L^{-1}), and the anaerobic threshold to peak (-0.5±0.8 mmol.L^{-1}, p=0.001) showed a significant drop in blood glucose and the ROC of blood glucose turnover. A significant drop in the magnitude in blood glucose from AT to peak can be explained due to the point where exercise intensity increases, there is a larger requirement in CHO as the predominant fuel, blood glucose utilization progressively increasing to meet demand, this fuel source must be replaced at an equal rate or blood glucose appearance will be reduced (Riddel et al, 2006). During intense exercise insulin secretion is inhibited by ß-cell α-adrenergic receptor activation. At this elevation in intense exercise glucose production rises seven to eight-fold and glucose production rises three to fourfold (Marliss & Vranic, 2002). To facilitate the changes in glucose delivery the increase in the glucoregulatory response is the increase in Glucagon, Growth hormone, cortisol and catecholamines to ensure adequate supply of hepatic glucose to the exercising muscles. However, despite this increase in counterregulatory hormones during intensity >80% in anaerobic exercise typically produces increases in blood glucose appearance in T1DM (Sigal et al, 1994), however, it could be argued that the importance of circulatory insulin to glucagon ratio in T1DM are the major determinants of glucose production and the necessity of the fall of insulin is needed for the full increment in both hepatic and glycogenolysis and gluconeogenesis (Wasserman et al, 2002). Furthermore, the combination of aerobic and anaerobic capacity during CPET which in hand can prevent the decrease in glucose production from aerobic work due to the increase in high intensity exercise which increases to production of blood glucose due to the shift in catecholamine (Adrenaline

and Noradrenaline) production (Marliss & Vranic, 2002, Wasserman et al, 2002) from a "feed forward" mechanism of hepatic glucose production by glycogen breakdown (Riddell et al, 2017, Kjaer, Farrell, Christensen & Galbo, 1986).

Furthermore, this coincides with other studies within CPET testing (Komatsu et al, 2005, Gawrecki et al, 2017) which demonstrated within CPET there was not any extreme variation in glycaemia to the point of hypoglycaemia/hyperglycaemia despite differences in types of insulin administration. However, there was no defined glucose values within these trials during exercise to evaluate any deviations in blood glucose within the CPET, as there was only a pre and post exercise blood glucose measurements. Also literature in analysing diabetic children (n=28) (Brouhard et al, 1985) through CPET which was devised through using a treadmill protocol and using a graded intensity every 3-minutes, which in hand showed a rise in blood glucose from pre-exercise 15.1±1.1 $mmol.L^{-1}$ to 18.1±1.2 $mmol.L^{-1}$ post-exercise.

This larger degree in the drop in blood glucose in comparison to our study could be due to the use of treadmill exercise due to it being more of a whole body exercise, also due to the 3 minute graded progression in intensity as this would increase the duration of the exercise and using more aerobic pathways for longer which has be shown to cause a larger drop in blood glucose in T1DM, rather than a more rapid increase in resistance to exhaustion. Furthermore, this could be due to the difference in the type of cohort chosen, as the use of children may have a significant change in glucose responses during exercise. However, other studies demonstrated the variation in BG using an ergometer but also testing teenage girls (n=19) with T1DM found that in comparison to resting blood glucose levels, were significantly lower after every 2 minutes until peak, whereas control healthy individuals blood glucose levels stayed constant (Heyman et al, 2007). However, due to the type of 2 minutely increase in intensity this again elongated the time the individuals were exercising, and using aerobic pathways for longer, which in hand can reduce blood glucose levels in T1DM. Therefore, this helps characterise blood glucose levels during CPET and the type of protocol can slightly differentiate the responses in glycaemia during exercise. Finally, this shows the efficacy of the CPET protocol when applied to around exercise and correct preparation in controlling blood glucose levels.

4.4. Glycaemic response in Euglycemia vs. Hyperglycaemia in CPET

This present study determined that there was no difference in exercise performance (Similar $\dot{V}O2peak$) and time to exhaustion. Niranjen *et al* (1997) demonstrated with groups of 'normoglycemia' or 'hyperglycaemia' showed a reduced $\dot{V}O2max$ and peak workload in the hyperglycaemic group, however the participants had been reported to have longer periods with higher HbA1c in their diabetes duration (≥6 years), which may have had more accentuated change in performance. Furthermore, studies proposing that performance during incremental exercise testing is compromised due to induced physical stress (higher rated perceived exertion) inhibiting the opioid endogenous system to respond to

this exercise induced stress (Riddell et al 2013, Wanke et al, 1996) and creating a higher perceived exertion in whole body exercise (Riddell et al, 2000). However, studies (Steller et al, 2006, Rothacker et al, 2021) showed that individuals under consistent hyperglycaemia (Clamp procedure) under CPET had no influence in performance by hyperglycaemia, with comparable glucose availability/oxidation or differences in power output compared to the Euglycemic group. Despite hyperglycaemia being consistent under clamp procedure, as this prevents the natural responses in blood glucose over CPET in T1DM unlike our study, and the lack of studies, which assess the response of starting blood glucose under CPET.

During CPET the significant rate of change and drop in blood glucose compared the euglycemic groups fell at a faster rate throughout CPET by understanding the drop in blood glucose levels and then this was divided by the overall time it took between the two points the drops occurred. Studies have shown due to the high availability in blood glucose in a hyperglycaemic state during exercise could be argued due to the partial suppression of hepatic glucose production via over riding feed-forward activation by elevated blood glucose levels (Howlett, Angus, Proietto & Hargreaves, 1998) to the point hepatic glucose production was slightly lesser than glucose utilization. Furthermore, it has been proposed that when being in hyperglycaemia this exaggerates the suppression of lipid oxidation during exercise, which could be, one of the leading factors to a larger decrement in blood glucose levels (Mascarenhas et al, 2016) as hyperglycaemia has been shown to prioritise persistent oxidation of CHO and intramyocellular glycogen is spared, respectively (Berger et al, 1977). Despite the significance in the drop in blood glucose in comparison to Euglycemic, this did not significantly drop in contrast to baseline blood glucose. Due to the increase in intensity, there is evidence to support that catecholamines responses to intense exercise are responsible for both glucose production (Sigal et al, 1999) and restrained increase in oxidative glucose utilization during hyperglycaemia in exercise in T1DM despite a larger glucose availability (Marliss & Vranic, 2002) as studies have shown that high blood glucose levels can acutely downregulate GLUT 4 and glucose clearance which limited glucose uptake (Mathoo, Shi, Klip & Vranic, 1999). Therefore, despite a tight control of blood glucose levels around CPET, exercising in hyperglycaemia can achieve similar performance outcomes.

4.5. Physiological and Metabolic Responses to CPET in Hyperglycaemia vs Euglycemia

During the period into peak exercise demonstrated a reduction in O_2 pulse under hyperglycaemia, this coincides with Baldi *et al* (2010) which showed poorly controlled glycaemia during exercise can elicit reductions in oxygen economy and extraction during peak exercise compared to normoglycemic individuals. Niranjan *et al* (1997) showed over 7-year trial period individuals under hyperglycaemia resulted in markedly impaired oxygen uptake under maximal exercise and display a reduced maximal workload. However, Gusso *et al* (2008) found no change in whole-body oxygen difference between

diabetes and non-diabetes individuals at peak exercise and only found a reduction in aerobic rate at submaximal, though this study did not evaluate glycaemic control for diabetic individuals to assess any atypical responses in blood glucose levels during exercise. Furthermore, there was not a thorough explanation in CPET protocol, which could explain some differences between our physiological responses due to the type of protocol used.

Further studies found influences in the change in O_2 availability due to the reduction in lung diffusion capacity at peak exercise in individuals under the influence of hyperglycaemia (Baldi et al, 2011, Naranjan et al, 1997, Villa et al, 2004). This suggested the limitation to exercise induced increases in the need for lung diffusion capacity and cardiac output which is affected from high blood glucose levels during exercise, therefore showing a reduction in stroke volume (Gusso et al, 2008) and a compensation for a higher heart rate (Baldi et al, 2010) as it has been established having higher blood glucose during exercise is inversely correlated to changes in heart rate variability (Singh et al, 2000). Therefore, demonstrating the impact in have higher starting blood glucose levels (hyperglycaemia) during CPET and the need for glucose stability.

A lower O_2 pulse might correspond with lactate levels during peak intensity and the recovery periods due to the shift in non-oxidative metabolism and a consistent stimulation of sympathetic activity and parasympathetic activity withdrawal (Stickland, Morgan & Dempsey, 2008). During CPET, individuals under hyperglycaemia demonstrated at peak exercise the rate of fuel metabolism (RER) was higher in carbohydrate utilization however this did not differentiate in performance output, this coincides with Stettler et al (2006) as they showed no change in V̇O2 max with euglycemia and hyperglycaemia in individuals who participated in two CPET under both starting glycaemic levels. Nonetheless, they discovered no change in RER, HR or lactate levels between the two groups. The differences in the metabolic biomarkers from their study could be due to the clamp procedure as this does not represent ‘real world’ physical exercise and glycaemia being maintain by artificial infusion of glucose. Furthermore, the use of CPET protocol is drastically differing from ours with a longer duration and a reduction in the rate of the increase in intensity, therefore this can propose a distinction between metabolic changes.

The change in RER at peak exercise could be explained with the increase in lactate production. This demonstrates the impact of starting glycaemia in CPET, as this suggests that the increased availability in plasma glucose does not increase intracellular glucose oxidation through oxidative pathways but through non-oxidative glycolytic pathways as hyperglycaemia has been shown to enhance glycolysis as intracellular glycogen is not spared for carbohydrate oxidation despite higher blood glucose availability (Jenni et al, 2008); Therefore, there is increased production of lactate (Lund et al, 2019) as it has been shown during intense exercise (>75% V̇O2 max), but not moderate exercise, similar

availability in substrate metabolism to non-diabetic individuals for carbohydrate utilization (Raguso et al, 1995).

Secondly, this could be due to the metabolic pathway blockage, as there are studies that show there is a reduced pyruvate dehydrogenase activity, therefore a decreased changeover of pyruvate to acetyl co enzyme (Bjorkman, 1986, Wahren, Hagenfeldt & Felig, 1975) which inhibits substrates entering the Krebs cycle and going through oxidative phosphorylation in the mitochondria. This in hand would increase the use of pyruvate through lactate oxidation for fuel metabolism. Finally, one metabolic factor that could have played a role in mediating substrate use could be due to the build-up of intramuscular glucose-6-phosphate (Wasserman, 1995), which could explain the reduction in glucose disappearance, and funnelling the use of these substrates for lactate production. The relatively small changes in metabolic responses in groups starting at different levels of glycaemia demonstrates the significant impact this has of clinical application in understanding the relevance of pre-exercise protocol in individuals with T1DM in CPET as this shows the impact of glycaemia on performance outcomes and the effects this has on metabolic function, respectively.

4.6. Limitations

Due to the lack of previous studies evolving around analysing glycaemia during CPET, more research would be required to produce a proper foundation in assessing the changes in Glycaemia during CPET. Furthermore, the use of the IPAQ questionnaire which is a self-reported questionnaire which may have lacked objectivity. Also, due to limitation in the heterogeneity and the wide range in Diabetes duration, HbA1c, at the start of CPET and Sex distribution.

4.7. Strengths

Firstly, the number within our study when comparing groups of individuals who started at hyperglyemia (n=10) to euglycemic (n=26) and overall (n=36) allowed us to effectively assess physiological variations in a suitable populations size. Also, the use of an effective and structure CPET protocol pre and post exercise to ensure an in-depth analysis of glycaemia during exercise to ensure quality data outcomes and safety of the individual.

4.8. Recommendations

When assessing individuals with T1DM in CPET it is important for the individual and the clinician to understand their glycaemia pre-exercise, to be educated in the physiological outcomes and the effects this has on glycaemia. Within cardiovascular rehabilitation clinics within T1DM it is important to assess starting glycaemia to accurately and safely produce an individual's data and to apply physical exercise prescriptions effectively by using $\dot{V}O_2$ max percentages instead of heart rate max to ensure safe and accurate prescriptions are applied.

4.9. Conclusion

This study explored the effect of CPET around acute glycaemia during progressive exercise testing to exhaustion to assess blood glucose changes during varying physiological stress. We found that under correct CPET protocol, glycaemia showed no significant overall change and did not result in hypoglycaemia within our cohort. However, starting exercise with high blood glucose levels (hyperglycaemia) impacts on some cardio-metabolic outcomes and can elicit a higher HR max and during recovery phases have a higher affinity for oxygen. Furthermore, performing exercise at maximal intensity whilst hyperglycaemia increase carbohydrate oxidation and a higher lactate concentration into recovery. Notwithstanding, performance capacity was not affected. Finally, our study demonstrates the safe implementation of a well-designed CPET protocol and appropriate glycaemic adjustments before exercise in T1DM.

4.10. Clinical Application of CPET in T1DM

From this study we have shown that during CPET in T1DM there are some metabolic variations with individuals under different starting blood glucose levels. This showed no significant changes in performance outcomes or reduction in power out over the exercise period. Therefore, this shows that when assessing T1DM individuals who start at different ranges in glycemia, performance outcomes will not be hindered in assessing the physiological responses under CPET, however it would be suggested for individuals to be within euglycemic levels prior to CPET.

Our results demonstrate that CPET can be performed safely in individuals with T1DM and used as an effective tool in assessing an individual's metabolism, cardio-respiratory and fitness levels within a clinical environment. From our results, finding that heart rate response at peak exercise can differentiate if the individual is starting at a higher blood glucose level from an increase in lactate accumulation and a lower O_2 saturation. We can use this in within cardiac rehabilitation for individuals with T1DM to make sure safe and effective preparatory protocol is used around CPET. Therefore, making sure appropriate pre-exercise adjustments are made and glycaemia is well controlled and stable before and during CPET which can help aid rehabilitation programmes and apply safe exercise prescriptions to an individual (Buckley et al, 2021).

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Appendices

Appendices

5.1. Example: Deriving data from individual data biomarkers

00:03:00	0.3
00:06:00	0.7
00:07:00	0.7
00:08:00	0.9
00:09:00	1.1
00:10:00	1.3
00:11:00	1.4
00:12:00	=AVERAGE(B142:B148)
00:13:00	
00:14:00	
00:15:00	2.3
00:16:00	2.7
00:17:00	3.0
00:18:00	3.2
00:19:00	3.4
00:20:00	2.4
00:21:00	1.5
00:22:00	1.2
00:23:00	0.8
00:24:00	0.7
00:25:00	0.7

AVERAGE(**number1**, [nu

00:10:40	1.474	1.2
00:10:45	1.322	1.2
00:10:50	1.352	1.2
00:10:55		
00:11:00		
00:11:05	1.427	1.3
00:11:10	1.552	1.2
00:11:15	1.578	1.2
00:11:20	1.661	1.2
00:11:25	1.618	1.2
00:11:30	1.455	1.3
00:11:35	1.332	1.4
00:11:40	1.621	1.4
00:11:45	1.598	1.3
00:11:50	1.778	1.4
00:11:55	1.678	1.4
00:12:00	1.692	1.4
00:12:05	1.783	1.4
00:12:10	1.561	1.4
00:12:15	1.742	1.4
00:12:20	1.937	1.
00:12:25	1.749	1.5
00:12:30	1.750	1.6
00:12:35	1.857	1.6
00:12:40	1.959	1.6
00:12:45	1.840	1.5

AVERAGE(**number1**, [numbe

5.2. Participant example: Derving data to calculate the ROC in glucose during CPET

PAT ID	BG EKF (mg/dl)	Change BG	minutes	ROC BG
3	10.67	0.13	3	0.04
3	10.8	-0.48	1	-0.48
3	10.32	-0.21	1	-0.21
3	10.11	-0.19	1	-0.19
3	9.92	0.29	1	0.29
3	10.21	-0.17	1	-0.17
3	10.04	0.51	1	0.51
3	10.55	=B10-B9	1	-0.09
3	10.46	-0.25	1	-0.25
3	10.21	0.05	1	0.05
3	10.26	-0.22	1	-0.22
3	10.04	0.07	1	0.07
3	10.11	-0.96	1	-0.96
3	9.15	-1.86	1	-1.86
3	7.29	1.84	1	1.84
3	9.13	0.26	3	0.09
3	9.39	0.09	3	0.03
3	9.48			

PAT ID	BG EKF (mg/dl)	Change BG	minutes	ROC BG
3	10.67	0.13	3	0.04
3	10.8	-0.48	1	-0.48
3	10.32	-0.21	1	-0.21
3	10.11	-0.19	1	-0.19
3	9.92	0.29	1	0.29
3	10.21	-0.17	1	-0.17
3	10.04	0.51	1	0.51
3	10.55	-0.09	1	=C9/D9
3	10.46	-0.25	1	-0.25
3	10.21	0.05	1	0.05
3	10.26	-0.22	1	-0.22
3	10.04	0.07	1	0.07
3	10.11	-0.96	1	-0.96
3	9.15	-1.86	1	-1.86
3	7.29	1.84	1	1.84
3	9.13	0.26	3	0.09
3	9.39	0.09	3	0.03
3	9.48			

5.3. Derving Glucose data for participants

Capillary earlobe glucose

Mins	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
0	10.67	8.58	6.88	7.42	9.62	7.06	6.10	8.63	5.36	5.46	11.31	6.40	8.71	6.90	6.40	9.17	6.53	7.24	10.71	8.84	9.59	10.89	6.72	9.13	8.31	12.27	7.08	6.99	10.39	13.83	6.44	6.30	12.33	14.30	13.16	13.23
6	10.80	8.12	7.19	7.72	8.07	6.92	6.02	8.49	5.35	5.34	11.41	7.01	8.43	9.04	8.03	9.86	7.50	6.87	10.88	8.85	9.72	10.80	6.70	9.10	8.29	12.38	7.01	7.16	10.30	13.53	6.30	6.14	12.30	14.12	13.25	13.13
7	10.32	8.17	7.43	8.18	9.07	7.09	5.80	7.95	5.19	5.38	11.47	7.23	8.92	9.32	8.28	9.70	7.76	6.70	11.16	8.86	10.11	10.63	6.56	9.15	8.28	12.61	6.95	7.44	10.28	13.41	6.21	6.04	12.21	13.85	13.35	13.08
8	10.11	8.06	7.72	7.88	8.29	6.69	5.94	9.16	5.23	5.28	10.68	7.49	9.08	9.34	8.34	9.96	7.75	6.89	10.94	8.77	10.02	10.77	6.52	9.41	8.17	12.18	7.03	7.06	10.45	12.95	6.20	5.98	12.34	14.14	13.18	13.28
9	9.92	7.99	7.51	7.63	9.51	6.49	5.88	8.72	5.53	5.42	11.11	7.56	9.14	9.51	8.72	9.78	7.95	6.78	11.28	9.06	10.08	10.88	6.43	9.27	8.19	12.42	6.89	7.44	10.30	12.97	6.13	5.92	12.46	14.17	13.18	12.99
10	10.21	7.88	7.95	8.19	10.17	6.80	5.81	8.96	5.51	5.41	11.19	7.61	9.03	9.44	8.70	9.95	8.02	6.78	10.79	8.78	10.34	10.61	6.38	9.50	8.42	12.08	7.02	7.11	10.34	13.03	6.21	5.88	12.23	14.27	13.21	13.03
11	10.04	7.86	7.61	7.18	9.37	6.74	5.92	8.66	5.30	5.44	11.26	7.83	8.53	9.67	8.83	10.16	8.27	6.60	11.14	9.18	10.27	10.74	6.43	9.33	8.33	11.98	7.01	7.05	10.41	13.09	6.24	5.68	12.43	14.12	13.22	13.13
12	10.55	8.05	7.52	8.03	9.61	6.75	5.75	9.13	5.38	5.37	10.76	7.17	9.47	9.38	9.08	10.02	8.52	6.28	11.22	9.00	10.40	10.74	6.22	9.39	8.39	11.65	6.94	6.84	10.38	12.65	6.12	5.79	12.45	14.18	13.30	13.43
13	10.46	7.91	7.71	7.51	9.48	6.80	5.75	8.93	5.38	5.37	11.54	7.86	8.97	9.34	9.36	9.87	8.56	6.06	11.21	9.03	10.34	10.42	6.16	9.38	8.20	11.86	7.09	6.63	10.41	12.51	6.11	5.49	12.60	14.50	13.28	13.02
14	10.21	7.72	7.58	8.20	8.30	6.79	5.71	8.60	5.07	5.26	11.30	8.08	9.33	9.28	8.83	9.95	8.75	5.96	11.02	8.89	10.75	10.37	5.98	9.47	8.38	11.44	7.00	6.57	10.53	12.23	6.07	5.44	12.41	14.28	13.41	12.72
15	10.26	7.82	7.98	7.39	9.48	6.75	5.81	8.00	5.04	5.17	11.30	7.95	9.35	9.13	8.82	9.66	8.61	5.93	10.37	9.16	10.32	10.37	5.88	9.37	8.46	11.47	7.01	6.10	10.32	11.97	6.01	5.20	12.27	14.68	12.96	13.13
16	10.04	7.74	7.84	7.98	9.37	6.64	5.44	8.49		5.15	11.53	7.86	7.94	8.93	9.34	10.13	8.62	5.76	10.49		10.60	10.08		9.33	8.52	11.41	6.87	6.84	10.61	11.70	6.08	5.19	11.97	14.29	13.59	12.81
17	10.11	7.95	7.67		9.26	6.94	5.63	8.40		5.16	11.19	7.78	7.72	8.62	9.66	9.65	8.37	5.27	10.66		10.81			9.40	8.23	10.81	6.78		10.62	11.23	6.12	5.22	11.92	14.25	13.33	12.58
18	9.15	7.88	7.56		9.60	6.59	5.42		4.81	5.04	11.29	7.66		8.56	9.77		8.35	4.93		11.24	10.57		5.42	9.57	8.44	10.77	6.79		10.81	11.06	6.25	5.09	12.36		13.47	12.62
19	7.29	7.86	7.41	7.75	8.80	6.56	5.36			4.78		7.42		8.25	9.31		8.30				10.84	9.62		9.59	8.23	10.27	6.73	5.50	10.73	10.91	6.33	5.05	12.43		13.43	12.63
20	9.13		7.35			6.43	5.33	8.57		4.68			8.81		9.60	9.61	8.12		10.20		10.89			9.50	8.39	10.17	6.62		10.83	11.09			12.26	13.88	13.07	12.28
21		7.94		9.14		6.32	5.12		4.47	4.59	11.19						8.67	5.33		10.92	10.63		5.32	9.54	8.44	9.83	6.83					11.48			
22	9.39			7.26		6.84	5.38			4.63		7.23	6.59	7.32			7.63				10.81	9.20		9.63		9.75		5.17			6.40	6.02				
23			6.34				5.22	6.67		4.53					9.83	9.25			9.11		11.18								11.75	10.77				13.99	14.02	12.68
24		7.83			9.10		5.17				10.80			3.74				4.52							9.71		7.38						10.63			
25	9.48					6.22	4.83					7.24					7.84							10.38		10.81					6.87	6.57				
26			6.34				5.05			5.55					9.76						10.80								12.08	10.82				13.94	12.35
27																									9.53		7.36						10.96			
28						5.58											7.00							10.28		10.53									
29							4.49			5.45											11.32															
30																																				
31							4.57																													
Sit	10.67	8.58	6.88	7.42	9.62	7.06	6.10	8.63	5.36	5.46	11.31	6.40	8.71	6.90	6.40	9.17	6.53	7.24	10.71	8.84	9.59	10.89	6.72	9.13	8.31	12.27	7.08	6.99	10.39	13.83	6.44	6.30	12.33	14.30	13.16	13.23
Warm up	10.80	8.12	7.19	7.72	8.07	6.92	6.02	8.49	5.35	5.34	11.41	7.01	8.43	9.04	8.03	9.86	7.50	6.87	10.88	8.85	9.72	10.80	6.70	9.10	8.29	12.38	7.01	7.16	10.30	13.53	6.30	6.14	12.30	14.12	13.25	13.13
Workload	9.90	7.92	7.68	7.80	9.29	6.69	5.54	8.66	5.33	5.13	11.21	7.69	8.98	9.21	9.00	9.92	8.29	6.27	10.96	8.97	10.32	10.61	6.34	9.41	8.33	11.30	6.91	6.92	10.48	12.29	6.15	5.58	12.31	14.25	13.30	12.96
Peak	9.13	7.86	7.36	7.99	8.80	6.54	5.05	8.40	5.04	4.53	11.39	7.42	7.72	8.25	9.60	9.85	7.83	4.93	10.66	9.16	11.18	10.06	5.88	9.63	8.44	#REF!	6.63	5.94	10.89	11.09	6.39	5.05	11.44	14.25	13.07	12.28
Cool down	9.39	7.94	6.34	7.75	9.14	6.22	4.49	8.57	4.81	5.55	11.19	7.23	8.81	7.32	9.83	9.61	7.84	5.33	10.20	11.24	10.80	9.62	5.42	10.38	9.71	10.81	7.38	5.50	11.75	10.77	6.40	6.02	10.63	13.88	14.02	12.68
End	9.48	7.83	6.34	7.26	9.10	5.58	4.57	6.67	4.47	5.45	10.80	7.24	6.59	3.74	9.76	9.25	7.00	4.52	9.11	10.92	11.32	9.20	5.32	10.28	9.53	10.53	7.36	5.17	12.08	10.82	6.87	6.57	10.96	13.99	13.94	12.35
AT	10.55	7.88	7.92	7.63	10.17	6.79	5.33	8.96	5.59	5.15	11.28	7.17	9.47	9.67	8.83	10.02	8.61	6.78	11.22	9.06	10.34	10.61	6.38	9.50	8.42	11.86	7.02	7.44	10.34	13.09	6.21	5.88	12.60	14.12	13.22	12.99

5.3.1. Individual data formulated for each biomarker

Mins	1
0	10.67
6	10.80
7	10.32
8	10.11
9	9.92
10	10.21
11	10.04
12	10.55
13	10.46
14	10.21
15	10.26
16	10.04
17	10.11
18	9.15
19	7.29
20	9.13
21	
22	9.39
23	
24	
25	9.48
26	
27	
28	
29	
30	
31	
Sit	10.67
Warm up	
Workload	=AVERAGE(B5:B17)
Peak	AVERAGE(**number1**, [n
Cool down	
End	9.48